HIPPOCAMPAL NEURON FIRING IN
GEOMETRICALLY DIFFERENT ENVIRONMENTS:
EVIDENCE FOR LONG-TERM, INCIDENTAL, AND
INCREMENTAL LEARNING

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Thesis submitted for consideration for the degree of Doctor of Philosophy

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ABSTRACT

When rats move around environments, hippocampal neurons fire in restricted portions of these environments. These neurons are thus called place cells, and are thought to provide the rat with a map-like representation of space, which can be used to guide behaviours such as navigation, and environmental discrimination.

Previous work has indicated that place cells can show different firing patterns in different environments (O'Keefe and Conway, 1978; Kubie and Ranck, 1983). This phenomenon is often referred to as “remapping” (Muller, 1996). Previous studies had shown different patterns of firing in differently-shaped walled environments (Muller and Kubie, 1987). In particular, place cell remapping was reported between square-walled, and circular-walled boxes (Quirk et al, 1992; Sharp, 1997).

The present study re-examined the remapping phenomenon in square-walled and circular-walled boxes under controlled conditions which did not alter the rats’ directional sense.

All the experiments conducted for this study showed that remapping did not take place on initial trials in the boxes: the firing patterns in the two boxes were similar. This basic result stands in contrast to that of Quirk et al, 1992 and Sharp, 1997. Further experiments showed that although place cell patterns were initially very similar across the two boxes, the patterns diverged with experience. In the time-series experiment designed to examine this issue carefully, the results showed a correlation between the amount of experience and the amount of remapping. The transition from
similar to remapped firing patterns was gradual. This is interpreted as evidence for incidental and incremental hippocampal discriminatory learning; the hippocampus learns to represent two boxes as different that it initially considers similar.

Further study showed that this acquired pattern-alteration was stable after a delay period of a month, during which the animals were not exposed to the testing environment. When re-tested the patterns showed similar levels of remapping to that obtained at the end of the time-series experiment. This is interpreted as important evidence for long-term storage of learned patterns. Other tests are described which were intended to probe the nature of the initial similarity, and subsequent divergence, of the place cell firing patterns. Also described is an attempt to show transfer of knowledge from one environment to another.

It is argued that the present thesis makes a contribution to our understanding of learning and memory processes in the hippocampus.
INTRODUCTORY OVERVIEW

This overview has two aims: to outline the ways in which the present thesis makes a contribution to hippocampal study; and to provide the background context in which the thesis was begun. The overview is intended to be succinct, and references to particular studies are not generally given except where those studies are of particular relevance to this thesis.

Hippocampal involvement in learning and memory

The hippocampus is widely thought to be crucially involved in learning and memory.

While its precise role in mnemonic processes is not agreed-upon, several kinds of approaches, including those based on surgical lesions, genetic manipulation, and pharmacology, have produced increasing support for the above almost-universal assumption: in essence, animals including humans with impaired hippocampal function have been shown to be impaired on tasks requiring specific learning and memory components.

Moreover, various levels of study of the hippocampus have also indicated a physiological basis for long-term learning processes, involving experience-dependent changes in connectivity between hippocampal synapses. The phenomenon of experimentally-induced, long-term potentiation of synapses (Bliss and Lomo, 1973) is increasingly well-understood, is agreed to be dependent on NMDA receptors and calcium signals, and is the most commonly accepted model for that component of the
hippocampal physiological learning process which involves synaptic change (Bliss and Collingridge, 1993).

Despite a great deal of unit-recording study of the hippocampus in the last 30 years, however, the extracellular recording approach has, arguably, convincingly demonstrated only relatively simple features of presumptive hippocampal learning processes, at the level of single cells.

**Unit-recording evidence of hippocampal learning?**

Strong evidence for hippocampal learning processes, that might help to explain aspects of hippocampal function suggested by other approaches, has been difficult to obtain. A guiding aim is for experience-dependent alterations in firing patterns to be demonstrated in ensembles of hippocampal cells, and for these altered firing patterns to be shown to be correlates of a learning process relevant to behaviour.

One problem is that studies generally examine hippocampal cell responses after presumed learning has taken place (Muller and Kubie, 1987; Quirk et al, 1992; Wood et al, 1999). Another problem is that the demonstration of altered firing patterns in hippocampal cells may simply reflect altered firing patterns in other areas which input to the hippocampus, and may be indicative of sensory and high-order system resets, rather than synaptic change in hippocampus. For instance, if hippocampal place cells fire in one environment in one pattern ("representation A"), and, almost immediately upon exposure to a second environment, fire in a different pattern ("representation B") (Bostock et al, 1991; Kentros et al, 1998), this might not involve learning at all. In the paradigms used in these studies, perhaps only the consolidation of the new pattern,
and not the emergence, itself, of the new pattern, reflects a learning process. Such an interpretation is supported by the recent demonstration that NMDA-receptor-blockers given at the time of exposure to a second environment did not prevent the emergence of a different pattern ("representation B") in that second environment, but only prevented the new pattern from being stable on the following day: such that yet another pattern was seen ("representation C") on re-exposure to the second environment.

In short, some experience-dependent alterations in place cell firing may not depend on synaptic change, whether in hippocampus or elsewhere. The demonstration of the learning process in these studies appears to be limited to creating stability in a representation. This is not to say that if change occurs rapidly, we can rule out the possibility that such change does not involve learning.

Two studies have produced evidence of experience-dependent change in hippocampal cells, somewhat suggestive of a learning process (Mehta et al, 1997; 2000). Running along linear tracks, rats' place fields were shown to develop asymmetries, and to increase in size, as running progressed. Impairing NMDA-function does block these experience-dependent changes (Mehta et al, 2000). However, it is far from easy to suggest a role for such changes in place fields, in terms of behaviour. This phenomenon does not seem to apply to place fields in open environments. And moreover, far more problematic for interpretation, the effect only lasts for a day. The system is found to have reset back to baseline upon entry to the track environments on the following day.
Unit-recording evidence of other features of hippocampal learning and memory

There has been little attempt to show, at the unit-recording level, that information learned in one context is transferable to that of another context, despite the fact that information-transfer is held to be a crucial feature of many theories of hippocampal function (including eg. Eichenbaum, 1994; Morris and Frey, 1997; O'Keefe and Nadel, 1978; Squire, 1994). Finally, there has been no attempt at all, to my knowledge, to provide unit-recording evidence to the effect that experience-dependent, learned, firing-pattern alterations are stable in the long-term (eg. a month).

The contribution of this thesis to hippocampal studies - learning and memory

This study can be seen as trying to fill in some of the above-outlined gaps in the specifically learning-and-memory aspects of hippocampal unit-recording studies. In particular, this thesis aims to provide simple and convincing evidence of:

1) Experience-dependent alterations in the firing patterns of place cells, which are the presumed correlates of an incidental and incremental hippocampal learning process. (There is no strong claim for the relationship between these firing-pattern-alterations and behaviour, but one possibility is that these alterations reflect incidental environmental discrimination.)

2) The transfer of knowledge from one context to another.

3) Long-term storage and retrieval of that which has been learned.

The main contribution of this thesis to hippocampal studies rests on the claims to have provided evidence for these mnemonic-related processes. Those aspects of the experiments which shed possible light on the formal nature of the hippocampal coding of environments are generally omitted or not much emphasised, in favour of focusing
on hippocampal learning and memory issues. Accordingly, any merit in the present thesis stands or falls with respect to these three claims, of which the second is perhaps the most vulnerable.

The study does present results, from all the experiments reported (Chapters 7, 8 and 9), which contradict a simple view that hippocampal firing pattern differences between a square and a circle are obligatory. As such, this is already a contribution to the field, since most workers appear to have assumed it was obligatory (see next section). But in my view the most interesting aspect of the thesis for hippocampal studies in general is probably that part most obviously concerned with learning and memory.

Stimulus and motivation for the present study
The present study builds upon previous studies of place cells in different environments, and it may be useful to comment on these, in so much as they help explain the original stimulus and motivation for the present study.

The studies of Muller and Kubie (1987), Quirk et al (1992), and Sharp (1997) had all found that markedly different place cell firing patterns occurred in walled-environments of different shape. In particular, the patterns shown in circles, on the one hand, and squares or rectangles on the other, were so dissimilar that the firing patterns seen in one environment were completely unpredictable on the basis of the firing patterns in the other environment. This pattern dissimilarity has also been seen in different-coloured environments, and is commonly referred to as “remapping” (Bostock et al, 1991; Muller, 1996).
In seeming contrast to these studies, O’Keefe and Burgess (1996) found regularities in firing patterns across environments of different shape. Four rectangular walled environments varying in the length of one or both dimensions were used. Place cells fired in a way that did not show “remapping”; the majority of across-shape firing patterns could be understood in terms of a relatively simple model which assumed that each cell fired in response to a fixed set of inputs. There was continuity, rather than discreteness, in the representation of the four environments.

A common, and probably the dominant, interpretation of all these studies, as represented by Muller’s (1996) review at that time, was that if environments were “sufficiently different”, they would induce hippocampal remapping. Implicit in this view was the idea that squares and rectangles were not sufficiently different from each other, but that circles and squares/rectangles were. Explicit was the notion that remapping occurred in an all-or-none fashion, instantaneously. The moment of remapping might be immediate, or delayed, with respect to the moment of exposure to a new environment, but if it occurred, all cells would change their firing patterns, as far as could be detected, *simultaneously* (Bostock et al, 1991; Kentros et al, 1998; Muller, 1996). More detailed discussion of the above-mentioned studies is deferred to the Discussion chapter.

Since squares and rectangles were apparently insufficient to induce remapping, the present author decided to re-examine the remapping phenomenon in square and circular-walled environments. The present thesis presents all the experiments carried
out by the author in relation to this task, including a brief chapter on the initial pilot studies (Chapter 7).
CHAPTER 1
HISTORICAL INTRODUCTION

A brief history of spatial mapping

This thesis is about the change with experience in the map-like representation of space in rats, a representation neurally instantiated in hippocampal cell firing patterns. Over time these cells learn to differentiate between environments they originally treated as equal. The thesis presents the representational change as reflecting incidental, rather than biologically motivated, learning processes. Once they occur, the changes persist for a long time, and are presumably used to guide future experience.

The thesis is thus about spatial maps, incidental learning, and the rodent hippocampus. How is it that these three things came to be associated with one another?

Temporal associative and representational accounts of learning

Psychology between and after the wars was sharply divided between two schools of thought about learning. The first and larger, which might be classed as "temporal associative", contended that learning took place as a result of the arbitrary associations between an organism's responses to the world, and what might be seen as the world's fixed and changing responses to the organism, ie. stimuli. These associations were often explicitly conceived to operate within a fixed framework of non-arbitrary, biological, and species-specific neural patterns corresponding to reflexes and drives. The nature of such drives was constant over an animal's life,
while a given drive's valency was reduced and increased according to the contingencies of living. While appreciating the importance of drives, the second and smaller school contended that animals could acquire the knowledge of representations of features and relationships in their environment (more will be said about this school later).

Associationists emphasised paradigms like classical and operant conditioning, conditioned reflexes, and stimulus-response chains (some of these terms are explained in what follows). The basic mechanism of learning operated through temporal contiguity, such as between an auditory tone, and shock, or between making the body turn rightwards, and seeing a food pellet. More concretely, synapses were neural site of the learning mechanism.

The flavour of such thinking can be savoured by quoting Allport on the conditioned reflex, in the context of stimulus-response theory:

"The prepotent reflexes are subject to synaptic change in their central portions. The effects of such changes are (1) to extend the range and complexity of the stimuli capable of exciting the response, and (2) to refine and specialize the response itself. The first effect, which may be called an afferent modification, is brought about by the principle of the conditioned response; the second, resulting in an efferent modification, is due to the selection and fixation of successful random movements in the processes of habit formation and thought".

(FH Allport, 1924)
In the temporal associative scheme, reinforcement related to the biological drives of a given animal (positive and negative), but since the learning mechanisms and many of the drives were assumed to be universal, potentially all behaviour might be explained in these terms. The associations themselves were, in contrast, arbitrary, potentially infinite, and coincidental. This school of thought viewed learning in explicitly anti-mimetic terms. Thus rats did not have formal models of the world, which they could manipulate as needed; they simply modified their behaviour patterns in response to its contingencies. Where certain strings of behaviour patterns were not prepotent, the law of effect “stamped in” random responses that led to reward, and stamped out those that led to punishment, or no reward.

With hindsight, the associative Behaviourists brought a great deal of rigour to psychology, and much insight is still available by continuing their programme carefully, but the narrow, exclusive manner in which their learning principles were applied now seems highly dogmatic.

With reference to learning in the spatial domain, and to rats in mazes, in particular, the temporal associative school conceived the solution of maze problems in terms of simple stimulus-response (S-R) connections. Tolman’s taxonomy of the two classes of thinking in the S-R school is useful. Tolman divided the different approaches to explaining how the rats arrived at the goals in mazes into:

1) Those which asserted that because “crucial stimuli from the maze get presented simultaneously with the correct responses more frequently than they do with any of the incorrect responses, [this greater frequency strengthens] “the neural
connections between the crucial stimuli and the correct responses...at the expense of the incorrect connections”.

2) Those which emphasised the backward temporal contiguity between the “need reductions” (ie. drive satisfaction) and the correct responses. In other words, “positive reinforcement”...strengthened the connections which have most closely preceded them”.

(Quoted from Tolman, 1948)

These kinds of ideas are, of course, still with us today. Thus in a recent computational model of spatial learning in the Morris water maze, locational, directional, and reinforcer cells are initially connected randomly, but when the model animal “encounters the reward location... connections are altered, so that recently active synapses are strengthened...thus, successful moves in a particular locational and directional context are “stamped in”” (Brown and Sharp, 1995).

But what if one were to demonstrate that rats could learn ostensibly spatial problems in the absence of reward, or in the absence of differential reward?

Latent, or Incidental learning

In opposition to this stimulus-response school, Tolman placed himself and others in a camp called “field theorists”. Tolman proposed that “in the course of learning, something like a field map of the environment gets established in the rat’s brain”, providing an explicitly mimetic representational account of how behaviour is determined.
Experiments on latent learning were clearly very important in causing Tolman and others to insist on the incompleteness of the simple associative story of rat maze learning behaviour. In his 1948 paper, Tolman adduced five kinds of experiments to support his cognitive field map theory, latent learning being the first, and perhaps most convincing, paradigm. Because it relates so directly to the present thesis, I concentrate on latent learning here.

A definition of latent learning might be that which occurs in the absence of reward, or in the presence of reward which does not differentiate between responses. Such learning was described as latent, in so much as the behavioural consequences of the learning were not demonstrated (or demonstrable) to experimenters until after reward had been introduced. The concept will be better understood by fleshing out the details of a crucial experiment by Blodgett (Blodgett, 1929).

Rats were run on a six choice-point alley maze (see Figure 1aA), with the furthest arm-end from the start-point containing the goal box, in which food could be placed. Rats were only run for one trial a day. For the 1st group of rats, the controls, food was placed in a goal box. For the 2nd and 3rd groups, food was only present in the goal box from day 3 to 7, and from day 7 to 9, respectively. (The experiment terminated on day 7 for the 1st and 2nd groups.) The control group’s errors declined linearly over days, while the errors of the experimental groups did not greatly diminish, until after food
Blodgett’s latent learning experiment

A) The 6 choice-point maze setup.
B) The learning curves for the three groups of animals

Figure 1a  The latent learning experiment of Blodgett (1929).

A) The 6 choice-point maze setup.
B) The learning curves for the three groups of animals
was introduced, at which point (day 4 for 2\textsuperscript{nd} group, day 8 for 3\textsuperscript{rd} group), the errors plummeted dramatically (see Figure 1aB). The errors for the 2\textsuperscript{nd} group on day 4 were indistinguishable from the control group on that day, and their error-curves merged from then. The 3\textsuperscript{rd} group’s error performance on day 8, having experienced food reward only once the previous day, was similar to that of the other groups on day 6. Similar results were seen in the times to traverse the maze. Finally, it needs to be said that on non-rewarded days, the controls were fed in the goal box and immediately again in their home cage, but the experimental animals were not fed until at least one hour later in a different environment that was not the home cage. Such a procedure minimises the role of purely temporal associations.

The most obvious conclusion to be drawn from these data is that the rats had been learning much more about the maze environment during non-rewarded trials than they had exhibited. Since this learning did not manifest itself until after reward had been introduced, Blodgett called it latent.

Tolman’s interpretation was that “as long as the animals were not getting any food at the end of the maze, they continued to take their time in going through it – they continued to enter many blinds”, (it is after all not necessary that such behaviour should attract the term “errors”), but once they were motivated to do so, they avoided blinds and went to the food directly. He concluded that during the non-rewarded trials they “had been building up a ‘map’”. (Tolman, 1948).

Tolman and Honzik performed a very similar study to Blodgett’s, on a 14 choice-point maze, feeding the non-reward animals two hours after reaching the goal, in their
home cages. They obtained similarly striking results (Tolman and Honzik, 1930, cited in Tolman, 1932, 1948). It is perhaps worth pointing out that Tolman and Honzik added a further control group who were never rewarded, but still showed a decline, admittedly modest compared to the other groups, in errors. It could be argued that they received a reinforcement at the goal box, of being taken away from the aversive presence of some lab stimuli, and the experimenters, to a more familiar cage. (Apparently Hull did in fact argue this point - O'Keefe, personal communication.) More recently however, Kavanau (1969, cited in Gallistel, 1990) attached a 512 choice-point vertical maze to the living quarters of mice; apparently the mice learned to traverse it without error, though no reward was present at the other end.

Such learning may perhaps more appropriately be called incidental, since it is patent to the experimenter. The term incidental learning will generally be preferred in this thesis, except where historically necessary. The important thing to stress at this stage is the unconditional, apparently obligatory, and “self-initiated” (Tolman, 1948) character of the learning in the experiments described above. Before leaving this section, it should quickly be emphasised that latent learning was explicitly applied by Tolman to situations in which reward was “non-differential [with respect to alternative responses] and very mild” (Tolman, 1948), but where rats subsequently showed, when motivated, that for instance they had acquired knowledge of the locations of food and water.

In the experiments described in the present thesis, rats are rewarded for moving around two similar but different environments. There is no differential reward
available to the rat which might encourage different overt responses, or representations, in the two environments.

Spatial orientation experiments – the detour

The final paradigm that Tolman selected to buttress the cognitive map concept involved what he called spatial orientation experiments. It is useful to describe the original and now classic, though flawed, sunburst experiment because it illustrates a form of latent learning, as well as suggesting directional constituents in the cognitive map.

An obvious criticism of theories of spatial behaviour which only allowed for S-R learning, without representational knowledge of environmental relationships, was implied in the question of what an animal might do if some of the stimuli in its environment changed. It is perfectly feasible to imagine that in a natural environment, a fallen tree might block a well-reinforced route normally taken by rats to a reliable food source. Strict S-R theory would predict an expensive series of trial and error journeys and/or frustration. Tolman, Ritchie, and Kalish (1946), in a paper called “Orientation and the short cut” asked if rats were capable of generating efficient detour behaviour. (The phrase “short cut” is evocative, but not entirely descriptive of their experiment.)

These authors first trained rats on an elevated maze which required the animals to walk on to a circular table, go up a straight alley, then turn through right-angled arms in a left, right, and right again sequence, to reach a goal box. Thus although the rats turned left or west out of the straight alley to eventually reach the goal, the goal was
located to the “north-east” of the straight alley. The intent was clearly to distinguish between a response or spatial strategy. Were the rats learning spatial information incidentally while they were learning to get to the goal box? Could they make a detour?

The test of this was to block the straight alley, attach a series of arms radiating from the circular table, remove the goal box, and observe which arms the rats entered. The results showed that after finding the straight alley blocked, and returning to the circular table, the rats “began exploring practically all the radiating paths. After going out a few inches only on any one path, each rat finally chose to run all the way out on one”. The path taken by the largest number of rats (36%) was indeed the one “which ran to a point some four inches in front of where the entrance to the food box had been” (Tolman, 1948).

(One problem with this experiment is that a light was placed above the goal arm. Arguably, the rats could have used a strategy involving, say, increasing the size of the light stimuli in their field of vision. As the finding has been replicated by others (eg. Harley, 1979) no further comment is made here.) Tolman’s interpretation was that rats had acquired a “map to the effect that food was located in such and such a direction in the room”, although there had been no requirement to do so for previously correct performance.

The “short cut” experiment and concept is at the heart of map-like incidental representational learning accounts. Such representations make for greater flexibility. While S-R models can be seen to account for some simple, ostensibly spatial
behaviours, they cannot deal simply with short-cut or detour findings, as is freely admitted for instance, by the authors of the water maze learning model, Brown and Sharp, quoted earlier. Hull's habit-family hierarchy concept, which tried to deal with such findings in an S-R way, had to posit "that the animal at the beginning of a maze experiment is already in possession of a vast repertoire of equivalent but fairly distinct locomotor habits, any one of which, in free space, would mediate a transition of his body from the starting point to the goal." (Hull, 1934). Like his assumption of transient reactive inhibition just after response-performance, in order to explain the spontaneous alternation behaviour of rats on T mazes, Hull's thinking seems to have had a high level of ingenuity in order to account for rats' map-ingenuousness.

At any rate, one of the important points for Tolman was that by acquiring knowledge of "field relations" (Tolman, 1932) in the environment (Tolman's understanding of which is mentioned in the next section), rats could use this information in a context different to that originally encountered. Thus, one of the key issues in cognitive map accounts is knowledge transfer, in the case of the "short-cut", between similar but different configurations of the same environment.

Tolman adduced other kinds of experiments in favour of the cognitive map view. We can partly illustrate his use of Muenziger's Vicarious Trial and Error (VTE) concept by simply referring to the hesitating behaviour in the sunburst maze quoted above, in which rats appear to be comparing the radial arms. In Tolman's view such behaviour illustrated that an animal's activity was not just of responding passively to discrete stimuli, in the way of an S-R machine lacking decision properties, but rather actively selecting and comparing stimuli. This kind of point was further elaborated with
respect to interesting work by Krechevsky showing so-called ‘hypothesis’ behaviour which seems to the human observer to demonstrate the successive use of different kinds of strategy to solve problems.

**Tolman’s “cognitive map”**

Our purpose to this point has simply been to indicate the type of finding that led to Tolman’s cognitive map formulation, and to emphasize the way in which latent learning, beyond the explanatory power of S-R models, is at the heart of the mapping concept. No attention has been paid to the huge variety of experimental evidence against S-R views of learning, such as those in the 1940s and 50s purporting to demonstrate place, rather than response learning. This section proceeds at once to flesh out Tolman’s map.

To anticipate somewhat, it is fair to describe O’Keefe and Nadel’s (1978) hippocampal cognitive map as far more restricted than Tolman’s, which was also unspecified neurally. O’Keefe and Nadel’s cognitive map involves an absolute spatial framework, imposed on the animal by pressures of evolutionary time, whereas Tolman’s map is relativistic, embracing many sets of relational properties which can expand in the lifetime of the animal. In many ways, Tolman’s ideas prefigure those of Eichenbaum (eg. 1994) as much as those of O’Keefe and Nadel. It is unnecessary, in my view, to suppose however, that Tolman’s map was entirely metaphorical, that he simply sought to suggest representational structures.
The 1948 paper gives little explicit detail of the mapping processes and structures. Certainly, it is clear that the map indicated "routes and paths and environmental relationships", and contained information "to the effect that" certain things were in certain places. Importantly, his language implies that rat maps are acquired in stages ("they had been building up a ‘map’"), and in a relatively autonomous way. It was also clear that rat maps were assumed to be more comprehensive than, for instance, maze area, and embraced "a wider arc of the environment" including spatial relationships inherent in the wider laboratory frame. Finally, as we have seen, he posited that the map could be used to determine spatial behaviour in a flexible way.

An important emphasis of Tolman’s (eg. Tolman, 1932, p176-178) was upon expectation: The map (using his later term) confers on the rat the ability to compare a current situation with a previously stored one. Tolman describes a thought experiment illustrating that the "perceptual expectation" derived from knowledge of "concrete spatial relations" could be the basis of disrupted behaviour and "surprise" if say, food was located in a different place from that in which it was orginally encountered (Tolman, 1932, p117). Thus some specific predictions about the role of learning and memory were predicated within the field map concept. It implied the map’s concrete spatial relations, which included in some way an indication of the disposition of objects, were subject to long-term storage, and could be retrieved as appropriate to generate efficient behaviour. Memory existed in the form of manipulable knowledge, which could be used to compare the current state of an environment and its features with an earlier state. This was an important and influential point of view. (O’Keefe and Nadel would later look for, and claim a neural basis for, such a function in the “misplace” comparator-type cells in the hippocampal CA1 field. It is also interesting
that from a different neuroethological perspective Ranck (1973) quickly found cells he later termed “mismatch” cells.

Tolman had comparatively little specifically to say about how the knowledge of the comprehensive set of relations in cognitive maps was organised into a dynamic system. And with regard to space itself, it is clear that his map concept lacked any fine detail on the formal nature of spatial representation. His sense that the field relations in the map were closely analogous to those of Lewin’s “Topologie” suggests a rather agnostic attitude towards geometrical ideas.

But we have seen enough of his ideas to show that they were not devoid of formal understanding, had predictive power, and were at least sufficient to inspire a search for neural codes of spatial relations. His theoretical emphases, with regard to spatial mapping, may now be summarised:

1) Mental maps embody spatial relations extractable from environments, such as distances, directions, sequences, and so on, within which the locations of objects can be stored.

2) During apparently “purely random exploring” (p.176), map knowledge is acquired unconditionally and relatively autonomously, for later use by the organism.

3) Maps provide flexibility – knowledge gained in one situation can be transferred out of that original situation to another similar, but different, context, to determine behavioural choices advantageous to the animal (eg. detour navigation).
4) Related to 3) The long-term storage of field relationships and objects within these confers, with retrieval, comparator properties to a cognitive mapping system, supporting expectations that guide more efficient behaviour.

These four principles show many similarities to axioms at the core of the O'Keefe and Nadel model of the hippocampal cognitive map. I hope to have shown how latent learning paradigms involve the essentials of all these four principles. Although Tolman's ideas were somewhat rough-and-ready, the conceptual stage was set sufficiently for future investigators.

Tolman's influence was probably both direct and indirect (through Hebb, for instance). The preface to O'Keefe and Nadel (1978) relates how Hebb's graduate seminar was particularly influential for the cognitive mapping theory. Hebb "emphasised the extent to which behaviourist notions could handle most of the available data and pointed to those few areas which showed the limitations of this approach: latent learning, sensory conditioning, the surprise of Tinklepaugh's monkeys". We have seen how two of these three areas, latent learning and surprise, had previously been mapped by Tolman. His sense of the specific role of a mapping system, and its particular contribution to behaviour was influential, particularly in the way he seized on Blodgett's latent learning and Krechevsky's "hypothesis" experiments. As we shall see, however, the formal nature of the spatial mapping system as proposed by O'Keefe and Nadel owed little or nothing to Tolman's scheme.
O’Keefe and Nadel’s hippocampal cognitive map


O’Keefe and Nadel turned to the philosophical and geographical literature, as well as psychological and neuroscientific study to tease out a highly specified model of the hippocampal contribution to spatial cognition. They contended that the hippocampal system’s workings generated the organism’s intuitive framework of absolute, unitary space. This framework was posited to be innate and Euclidean. The geomimetic quality (at least on a scale appropriate to a rat, for instance) of such a spatial representation was evolutionarily determined, and inescapably imposed on the animal. This is a remarkably Kantian position. (More recently, O’Keefe, (1996) has suggested that our representation of space is confined to a mere three dimensions due to the restrictions implicit in hippocampal structural anatomy.) The explicitly Euclidean framework stood in contrast to Tolman’s non-committal geometry, and the still-prevailing notions of relativistic, behavioural space.

The most important difference from the schemes of Tolman and almost all others (eg. Piaget) was their Kant-like insistence on the logically primary status of places. Tolman for instance had emphasised relativistic space constructed secondarily through processing relationships between objects, routes, combinations of routes and so on. Whether spatial representation can involve a preexistent framework
interpreting sense data obligatorily spatially, or is built-up from object-based, relativistic constructs, remains highly controversial today.

It seems that it was both the combination of the discovery of “place” cells, and philosophical considerations about space, that prompted O’Keefe and Nadel to stress the a priori nature of space. O’Keefe and Nadel explicitly adopted a Kantian position, positing the a priori nature of abstract places, represented by place cells:

“The constituents of space are places, and thus an alternative definition of a map is the representation of a set of connected places which are systematically related to each other by a group of spatial transformation rules. … The absolute space defined by Kant exists in the absence of objects.” (O’Keefe and Nadel, 1978, p.78).

Specifically, they proposed the hippocampal pyramidal neurons represented the places, that an as-yet-unidentified source of directional information existed in the hippocampus, and they speculated how speed and/or distance information could be generated for the workings of the mapping system. While compass-like direction cells have subsequently been found in the nearby presubiculum (Ranck, 1984; Taube et al, 1990), it remains unclear how distance might be coded in the system (see chapters 3, 4, and 5). There are very preliminary indications of speed cell signals in the hippocampus (O’Keefe et al, 1998) and the presubiculum (Sharp, 1997; Cacucci, Lever, and O’Keefe, unpublished), and there are stronger indications that place cell firing is modulated by speed (eg. Czurko et al, 1999, discussed in Chapter 4). At any rate, they presumed that the information was available to the mapping system to
systematically relate the places to each other by transformation rules embodied in the hippocampal neuronal network, and to provide the basis of an allocentric spatical system for navigation, environmental discrimination and so on.

Such assumptions were the basis of a theory which reinterpreted the existing hippocampal-lesion literature, and many made specific predictions for future study. This chapter ends by listing some of those predictions related to the present study. They are treated in more detail in the Discussion (Chapter 10), and are only briefly outlined here.

1) The hippocampus learns in a one-trial manner, and its maps are not incrementally altered by repeated exposures to the same environment.

2) The hippocampus both constructs and stores maps. Thus at least place information is stored in the long-term by the hippocampus.

3) The hippocampus is at least in part an incidental learning system. This is one of its differences from other learning systems in the brain. (These authors of course wished to make the point that the learning is not incidental in an ordinary sense, but reflect obligatory functioning of hippocampus in map-making.)

4) The hippocampus can retrieve information learned, in other contexts not identical to those in which the information was learned. This is the point about knowledge-transfer.

5) The hippocampus is exclusively responsible for generating exploratory activity, where this is understood to be a species-specific behaviour pattern related to information-gathering.
CHAPTER 2
ANATOMY OF THE HIPPOCAMPAL FORMATION

1) The hippocampal formation: Gross morphology, speciation, and intrahippocampal circuitry

The hippocampus proper is generally considered to consist of the dentate gyrus and Cornu Ammonis fields (CA3, CA2 and CA1) fields, while terms like “the hippocampal formation” also include one or more of the subiculum, entorhinal cortex, parasubiculum, and presubiculum. Some authors include the subiculum in “hippocampus”, since the subiculum is allocortical (having only three layers like dentate gyrus and CA fields).

Figure 2a is a sketch depicting where the hippocampus is in the rodent brain. This figure gives some idea of its position in relation to some key landmark structures like the septum and cerebellum, and anterior commissure and corpus callosum. It can be seen that the hippocampus (Figure 2a, shaded) represents quite a large portion of the rodent brain. The hippocampus proper represents about half the cortical volume of the rat. Figure 2a draws attention to the septal (or dorsal or anterior) and ventral (or roughly posterior) poles of the hippocampus. As mentioned below, these poles are associated with different connectivity. Further, Chapter 4 describes a study (Jung et al, 1994) suggesting that ventral hippocampal place cells have larger place fields than those found in the dorsal hippocampus. In the mammalian brain, the distinctive appearance of the hippocampus is derived from the interlocking of two cell-sheets which have curved to form U shapes. Figure 2b gives some indication of the two
Figure 2a  The relationship between the hippocampus and other major brain structures.

The left hippocampus has been exposed by removing the overlying cortex and all forebrain structures apart from those at the midline. The hippocampus is shaded to give an impression of its three-dimensional structure. (After O'Keefe and Nadel, 1978).
Figure 2b  A sketch of the major regions of the hippocampus, including the principal synaptic connections. Based on the anatomy of a transverse section through the ventral hippocampus (Paxinos and Watson, 1986). The perforant path input to the dentate gyrus targets the outer 2/3rds of the molecular layer.
interlocking U sheets, one made by the dentate gyrus, the other by the CA3 and CA1 fields.

With possible differences along the septal-ventral axis in rodents in mind, it is important to state that the evolution of the brain in primates including man has meant that in primates the hippocampus is found only more ventrally, a deep structure within the medial temporal lobe. The equivalent of the dorsal hippocampus in rats is the posterior hippocampus in primates. There is an emerging suggestion that the dorsal hippocampus in birds and rodents, and the posterior hippocampus in primates may preferentially be involved in tasks relating to spatial navigation (Colombo et al, 1998; Hock and Bunsey, 1998; Maguire et al, 2000; Moser et al, 1993; 1995), though suggestions of discrete functions in the two areas have not been convincing.

The increase in size of the hippocampus over evolution in primates to man has only been modest. Stephan and collaborators, in various studies cited in Eccles (1989), compared species and used an encephalization index taking account of size, using Tenrecinae, the most primitive living mammals, as a baseline (1.0 all areas). This results in an index of only 4.9 for the human hippocampus, which may be compared to thalamus (14.8), cerebellum (21.8), striatum (22.0), and of course the spectacular neocortex (196.4). The figure 4.9 does disguise some variety. The indices for dentate (2.8), subiculum (3.3), and CA3 (1.7) are all smaller than this average, while the CA1 index is 6.6. (Virtually all the cells in the present study were recorded from CA1).

Intrahippocampal circuitry
An overview of intrahippocampal circuitry is given here. Hippocampal connectivity differs from that of neocortex by lacking much of the strong, reciprocal innervation seen for instance in visual cortex. Hippocampal connectivity is much simpler, and more serial. Figure 2b sketches the principle synaptic connections involving the hippocampus proper, from:

1) Entorhinal cortex (layers II and III) to Dentate gyrus, CA3, and CA1 (via the "perforant path")
2) Dentate gyrus to CA3 (mossy fibres)
3) CA3 to CA1 (Schaffer collaterals)

If we add that CA1 projects to the subiculum and entorhinal cortex (deep layers), and that CA3 has quite dense associational inputs to itself, this picture gives us the basic elements in the circuit involving the hippocampus proper.

Some nomenclature and further details of connectivity may be mentioned by looking further at Figure 2b.

It may be seen that CA1 is divided into:

a) stratum oriens towards the alveus,
b) stratum pyramidale, the layer of the principal pyramidal cells,
c) stratum radiatum beneath the pyramidal layer (i.e. towards the hippocampal fissure), and
d) stratum lacunosum-moleculare beneath the stratum radiatum (i.e. the layer nearest the hippocampal fissure).
CA3 is similar, but is considered to have an additional narrow layer, the stratum lucidum, above the stratum radiatum. The stratum lucidum of CA3 is occupied by the mossy fiber axons originating from the dentate gyrus.

The stratum radiatum of the CA region can be defined as the region in which the CA3-CA3 associational and CA3-CA1 Schaffer collateral connections are located (Amaral and Witter, 1995). The dense perforant pathway fibers from neurons of the superficial layers of the entorhinal cortex course through the stratum lacunosum-moleculare, and terminate here, as do afferents from other regions, including the nucleus reuniens input from the thalamus.

The dentate gyrus consists of three layers. The stratum granulosum is the principle cell layer of the granule cells. The stratum moleculare is the layer closest to the hippocampal fissure, where the granule cell dendrites are located. Granule cells are unipolar - there are no granule cell dendrites on the other side of the granule layer. The dense projection to the dentate gyrus from entorhinal cortex targets the granule cell dendrites in the molecular layer. The third layer is the hilus, often called the polymorph layer of the dentate gyrus (Amaral and Witter, 1995).

2) Afferents to the hippocampal formation

Perhaps the major source of input to the hippocampus comes from the superficial layers (II and III) of the entorhinal cortex. Thalamic input via the entorhinal cortex may have been underemphasised, but it seems relatively certain that entorhinal cortex is the major route for neocortical information to reach hippocampus. Much of this entorhinal input to hippocampus comes in turn from postrhinal (rodent equivalent of...
parahippampal cortex) and perirhinal cortices, but all these rhinal cortices project directly to hippocampus.

This section considers first the cortical afferents to the rhinal cortex, then the afferents to the entorhinal cortex in general, and finally subcortical and other afferents.

**Cortical afferents to rhinal cortex**

Burwell and Amaral’s studies (1998a, 1998b) have shown interesting features of cortical input to the perirhinal (areas 35 and 36), postrhinal, and entorhinal (lateral and medial) cortices. All these cortical regions (considered as 5 regions) receive most of their inputs from unimodal association and polymodal association areas, suggesting that the hippocampus receives abstracted, highly-processed information from neocortex, but there is also some specialization of inputs associated with each cortical area. Not all will be described, but for instance:

1) postrhinal cortical input is 40% visual and 20% visuospatial, and less than 15% of its input comes directly from sensorimotor or primary unimodal areas. In fact, 71% of its input is from polymodal association areas.

2) lateral entorhinal input is 34% olfactory, and less than 7% of its input comes directly from sensorimotor or primary unimodal areas.

3) medial entorhinal input can be considered to be a kind of mixture of 1) and 2). Medial entorhinal input is 31% olfactory, 12% visual, 16% visuospatial (only postrhinal input has higher percentages for the visual/visuospatial input), and less than 16% of its input comes directly from sensorimotor or primary unimodal areas.
Para/Presubiculär input to Hippocampus mainly via Entorhinal Cortex

Medial Septum/Diagonal Band of Broca
Thalamus (Laterodorsal, Anterior, Reuniens, Intralaminar)
Basolateral Amygdala
Clastrum
Subiculum and CA 1

Parasubiculum
Presubiculum
Entorhinal Cortex
Layer II
Entorhinal Cortex
Layer III
Dentate Gyrus
CA 3
CA 1

Figure 2c. Diagram of the presubiculär and parasubiculär input to hippocampus, which occurs mainly through the entorhinal cortex. Parasubiculär input targets earlier, presubiculär input targets later, processing stages of the hippocampus.
All sensory modalities feed into these regions, though there is comparatively little input from gustatory areas, and auditory areas (except to area 36: 9%).

These general principles apply, *mutatis mutandis* (particularly with respect to olfaction), to the monkey brain. The authors give very convincing evidence that the postrhinal cortex can be considered homologous to the primate parahippocampal cortex.

We now focus on entorhinal, parasubiclar, and presubiclar input. The broad trends of neocortical input to entorhinal cortex have been described, with reference to Burwell and Amaral's studies (1998a, b). These authors ignored presubiclar and parasubiclar input in their studies (percentages given above did not take these areas into account). It has become increasingly obvious, however, that these areas are of particular importance to hippocampal function, and recent studies have increasingly paid attention to connectivity of the presubiculum and parasubiculum.

The next section describes the connectivity of these two related regions. Figure 2c is a diagram summarising the main points of this section.

**Parasubiculum and Presubiculum: Inputs via Entorhinal cortex**

We begin with the parasubiculum. Anatomically, it may be considered an input structure to the hippocampal formation (Amaral and Witter, 1995), and its efferents have received some systematic study. The parasubiculum densely and preferentially innervates medial entorhinal and lateral entorhinal area layer II cells, which project in turn, massively, to the dentate gyrus and CA3 (Caballero-Bleda and Witter, 1993,
1994; Kohler, 1985; Steward and Scoville, 1976; Van Groen and Wyss, 1990a; Witter, 1993). Additionally, the parasubiculum has a direct and fairly substantial projection to the molecular layer of the dentate gyrus (Kohler, 1985; Witter et al., 1988).

It can thus be seen that the parasubiculum targets the “earlier” stages of hippocampal processing. These early stages of hippocampal formation processing may be more strongly influenced by parasubiculum than previously thought, as suggested by a recent study estimating neuron numbers in the area: in the rat, parasubiculael external lamina (layer II/III) neurons ($1.5 \times 10^5$) outnumber those in layer II of the MEA and LEA combined ($1.1 \times 10^5$) for instance; together, the presubiculum and parasubiculum contain the same number of neurons ($7 \times 10^5$) as the MEA and LEA combined ($6.9 \times 10^5$), parasubiculum contributing about 30% to this total (Mulders et al., 1997). (Underestimation of the size of the parasubiculum may partly be due to the mapping of Swanson et al. (1978)).

The present author has found that parasubicular input (focusing on its caudal region) comes primarily from, subcortically: the medial septum/diagonal band of Broca complex (hereafter “medial septum”), anterior, reuniens, intralaminar, and laterodorsal thalamic nuclei, basolateral amygdala, and claustrum, and cortically: subiculum, entorhinal, visual and anterior and posterior cingulate cortices (Lever, Owen, Donnett, O’Keefe, unpublished). Rostral parasubiculum appears to have a larger input from CA1 and amygdala (Van Groen and Wyss, 1990a.) Input from visual and cingulate cortices was modest, and by far the greatest cortical input was
shown to be the subiculum. This suggests some serious scope for “reentrant” or “looping” circuitry in the hippocampal system.

There has been much interest in presubiculum since the discovery of head-direction cells (described in some detail in chapter 4) in this region (Ranck, 1984; Taube et al, 1990a). Head-direction cells with similar properties to those in presubiculum have also been found in anterior thalamus (Taube, 1995). There is some debate about presubicular cortex. Some have insisted on a division between postsubiculum and presubiculum (Swanson et al, 1978; Van Groen and Wyss, 1990a, b, 1992, 1995). The confusion is exacerbated by the general misidentification of Haug’s area 29e (Haug, 1976). I will treat afferents to the single region “presubiculum”.

Presubicular connectivity is similar to that of parasubiculum, except that the set of presubicular afferents is larger, and includes major input from visual and retrosplenial cortices, and the anterodorsal thalamus (Van Groen and Wyss, 1990a, b; 1995). It is likely that these last-three mentioned inputs contribute to the head-direction signals.

A difference from parasubiculum that is potentially important functionally is that presubicular output is primarily directed to layers III and I of entorhinal cortex, and to medial entorhinal cortex only. Layer III cells of entorhinal cortex project primarily to CA3 and CA1 of hippocampus. Accordingly, there is a pattern whereby, through entorhinal cortex, parasubiculum targets early stages of hippocampal processing (DG and CA3), and presubiculum targets later stages (CA3 and CA1). The functional meaning of the entorhinal layer II vs layers III/I segregation is unknown, and ought to be perceived as a challenge to the field. It is of interest that the perirhinal input to
entorhinal cortex also targets the presubicular preferential field in layers III/I of entorhinal cortex.

Since: a) there is no direct presubicular projection to hippocampus proper; b) the anterior thalamus projects primarily to deep layers of the entorhinal cortex (Shibata, 1993), and c) there is no input from anterior thalamus to hippocampus (except to layer I of subiculum: Van Groen and Wyss, 1995) it seems likely that the hippocampus primarily receives the thalamic head-direction signal via the presubiculum via entorhinal layer III.

If one had to summarise the specific contribution of pre- and parasubicular input to the hippocampal system, one might say that these regions channel in thalamic input via the superficial layers of entorhinal cortex, and may also provide a way for hippocampal output to be reprocessed as input. Presubicular input appears to be more obviously visual and visuospatial.

Interestingly, preliminary work has identified a category of cell, combining direction and place information, in the superficial layers of pre- and parasubiculum (Cacucci et al, 2000; Sharp, 1996). It is known that cells in these layers make synapses onto entorhinal-hippocampal projection neurons (Caballero-Bleda and Witter, 1994).

**Other subcortical input to the hippocampal formation**

Four major subcortical inputs to the hippocampal formation, not including thalamus, come from the medial septum, supramammillary hypothalamus, brain stem, and amygdala. The first three may be related to motor and arousal information. One of the
most important inputs to the hippocampal formation, which may act as a pacemaker in
the generation of the theta oscillation, is from the medial septum. Septal fibers
terminate in basically all areas of the hippocampal formation, and are particularly
prominent in the dentate gyrus (Amaral and Witter, 1995). The termination patterns of
the supramammillary nuclei are much more specific, and target the dentate gyrus and
CA2 in particular (Amaral and Witter, 1995). One speculation is that this input helps
provide speed-related information. Monoaminergic input from the brain stem is
primarily restricted to noradrenergic and serotonergic input. Noradrenergic input is
quite dense to all regions of hippocampus proper and subiculum, while serotonergic
input is densest to lateral entorhinal, dentate gyrus, and layer I of pre and
parasubiculum (Amaral and Witter, 1987). Basolateral Amygdalar input to
hippocampus is largely restricted to CA1 (Krettek and Price, 1977), and to its
temporal third (Amaral and Witter, 1995). Finally, it should be added that CA1
receives a direct and massive input from the nucleus reuniens of the thalamus
(Wouterlood et al, 1990), largely directed at the stratum lacunosum-molecular and to
CA1's middle septo-temporal levels.

Input to Septal vs Temporal hippocampus

It is not really possible in a summary of this nature to give an idea of all the septal-
temporal axis differences that exist with respect to hippocampal input. Extensive
studies (reviewed in Witter, 1986) have long shown that these do exist, and Witter
proposed that the septal hippocampus is more involved in the processing of sensory-
related information whereas the temporal hippocampus is more involved in visceral-
related processing. It is fair to say that more recent anatomical studies have tended to
confirm this general principle. Hormone-related, and neuromodulatory substances
and/or receptors have been found to be more dense, or simply existing only, in temporal hippocampus (many references cited in Jung et al, 1994). There appears to be almost no exception to the rule that where a concentration gradient exists for transmitters, neuromodulators, or receptors, it is denser towards the temporal pole.

3) **Efferents of the hippocampal formation**

**CA1 efferents**

CA1 gives rise to substantially greater extrinsic connections than the other CA fields (Amaral and Witter, 1995), and only those of CA1 will be described. Much of its output extrinsic connectivity is similar to the subiculum’s (described below). The following distillation is based on Van Groen and Wyss (1990c) and Amaral and Witter (1995). The most prominent projections to cortical areas are to medial prefrontal cortex, retrosplenial cortex, and perirhinal cortex. Important subcortical projections include those to the medial and lateral septum. Ventral CA1 includes projections to the anterior olfactory nucleus, olfactory bulb, nucleus accumbens, basal amygdala, and anterior and dorsomedial hypothalamic areas.

**Subicular efferents**

The subiculum is one of the major output regions of the hippocampal formation. Prominent projections of the subiculum include those to:

a) portions of medial prefrontal cortex, particularly to the medial and ventral orbitofrontal cortices, and to the prelimbic and infralimbic cortices

b) retrosplenial cortex

c) perirhinal cortex

d) medial anterior olfactory nucleus
e) lateral septum

f) nucleus accumbens, particularly its caudomedial part

g) ventromedial hypothalamic nucleus

h) medial mammillary nuclei (the lateral mammillary nucleus, important in generating the directional signal in anterior thalamus and presubiculum is only sparsely innervated by the subiculum)

i) medial thalamic nuclei including the nucleus reuniens, and interanteromedial nucleus


Entorhinal efferents: cortical

Deep entorhinal layers receive input from CA1 and subiculum; accordingly neurons in these entorhinal layers may be very important in acting as the hippocampal conduits to the rest of the cortex. There has been some debate, however, about whether or not the entorhinal cortex of the rat, like that of the monkey (Van Hoesen, 1982), gives rise to prominent and widespread projections to the unimodal and multimodal association cortex (Amaral and Witter, 1995). Swanson and Kohler (1986) suggested that rat entorhinal cortex gives rise to projections reaching a large domain of the cortical surface, while others suggested that only a very dorsolateral part of the entorhinal cortex projected to widespread neocortical areas (Sarter and Markowitsch, 1985, Insausti et al, 1994 cited in Amaral and Witter, 1995), and it was possible that this region was in fact perirhinal. A detailed study by Insausti et al
(1997) reexamined this issue. It is useful to quote directly both their findings and interpretation:

"Neurons in layer V of an extremely laterally located strip of entorhinal cortex, positioned along the rhinal fissure, give rise to the projections to lateral frontal (motor), parietal (somatosensory), temporal (auditory), occipital (visual), anterior insular, and cingulate cortices. Neurons in layer V of the most caudal part of the entorhinal cortex originate projections to the retrosplenial cortex. [...] Our results show that in the rat, hippocampal output can reach widespread portions of the neocortex through a relay in a very restricted part of the entorhinal cortex. However, most of the hippocampal-cortical connections will be mediated by way of entorhinal-perirhinal-cortical connections. We conclude that, in contrast to previous notions, the overall organization of the hippocampal-cortical connectivity in the rat is largely comparable to that in the monkey." (Insausti et al, 1997)

Entorhinal efferents: subcortical

The entorhinal subcortical efferents overlap those described for the subiculum and CA1. Like these two regions, but not the para- and presubiculum, entorhinal cortex projects to the septum, primarily to lateral septum (Alonso and Kohler, 1984). Entorhinal cortex also projects widely to the amygdala, especially to the basal nucleus. Other important efferents are those to the nucleus accumbens and olfactory tubercle. There have been no reports of entorhinal projections to the thalamus or brain stem (Amaral and Witter, 1995).

Parasubicular and Presubicular efferents
These regions may not be very important outputs for the hippocampal formation as a whole. As described above, the parasubiculum serves primarily as an input to the hippocampal formation. It has virtually no efferents to regions outside the hippocampal formation, except to those regions in thalamus which project to the hippocampal formation (e.g., anterodorsal thalamic nucleus) (Van Groen and Wyss, 1990a). The presubiculum has a wider set of efferents. These are predominantly to hippocampal formation, but include projections to the retrosplenial and perirhinal cortices, and to regions of thalamus that project to presubiculum (anterodorsal and laterodorsal thalamus). An important point is that presubiculum, via the fimbria/fornix, projects ipsilaterally to the lateral mammillary nuclei. Directional cells have been found in these nuclei, and it is likely that this represents a feedback loop.
CHAPTER 3

PHYSIOLOGY OF THE HIPPOCAMPUS

This brief chapter summarises physiological aspects of the hippocampus proper. The first part looks at states of the global hippocampal electroencephalogram (EEG), with a particular focus on the quasi-sinusoidal rhythm in the EEG known as theta.

Characteristics, generation, and correlates of theta are described. The second part considers cell types in the hippocampus, focusing on complex-spike firing in particular. Finally, the phenomenon of long-term potentiation in the hippocampus is discussed. Where relevant, relationships between physiology and behaviour are explored. (Chapter 4 reviews the literature on place cells.)

1) EEG states

Various EEG states have been described in the hippocampus. The most basic distinction is between theta (also called rhythmic slow activity by Vanderwolf (1969), and Large Irregular Activity (LIA: Vanderwolf, 1969). Vanderwolf also described a Small Irregular Activity state, characterised by a desynchronised, high-frequency, low-amplitude pattern. Most work has focused on LIA and theta. More recently, the higher-frequency gamma oscillations have been described in the rat (Bragin et al, 1995). LIA, gamma, and theta are briefly characterised, and then theta is considered in more detail.

LIA

Vanderwolf (1969) used the term LIA to describe the large-amplitude (1-3mV), irregular, slow waves in which the dominant frequency is slower than in theta, and which, with some electrode placements, contain sharp spikes of 40-100 ms duration.
Chrobak and Buzsaki, 1994; O'Keefe and Nadel, 1978). Buzsaki and others refer to these EEG spikes as sharp waves. Sharp waves are best seen in electrodes below the CA1 pyramidal layer, in stratum radiatum (O'Keefe and Nadel, 1978). It is thought that sharp waves result from the excitation of the apical dendritic field of CA1 neurons by their CA3 Schaffer collateral input.

Although the LIA phenomenon is aperiodic, there is a sinusoidal EEG state which is intimately associated with the large sharp waves. These are the “ripples” described by O'Keefe and Nadel (1978): small sinusoidal wave bursts where each wave has periods in the range 4-8 ms, with about 4-10 waves in each burst. (I tend to find that the majority of waves are at the lower end of the range reported by O'Keefe and Nadel (1978), generally having about 5 ms periods.) Ripples can be seen above, in, and below the pyramidal layer. As reported by O'Keefe and Nadel, the amplitude of ripples is maximal in or just below the pyramidal layer. The sharp waves themselves are positive or positive/negative in the stratum oriens, and negative, or negative/positive in the stratum radiatum. (All, these LIA-related observations can be used to guide electrode placement. See Methods, Chapter 6.)

Firing of hippocampal neurons appears to be disinhibited during LIA; complex spike cell bursting is seen coincident with the sharp waves, if electrodes are in the pyramidal layer.

The LIA EEG state only occurs during automatic, non-displacement behaviours such as sitting quiet, slow-wave sleep, eating, drinking, and grooming. The sharp waves occur most frequently during slow-wave sleep and quiet sitting, less frequently during
eating and drinking, and least of all during grooming; they are often inhibited by
arousing stimuli, even when no movement occurs (O'Keefe and Nadel, 1978).
Recently (Bragin et al, 1999) ripple states have been described in the human.

Buzsaki (1989) has suggested a model of memory trace formation in the hippocampus
in which the sharp waves, during rest or slow-wave sleep, play a major role in
potentiating memory traces of information acquired during exploration (the theta
state).

**Gamma frequency oscillations**

More recently, Buzsaki and colleagues have described gamma oscillations, ie. a low-
amplitude, fast 40-100 Hz frequency activity, in the hippocampus of the awake,
behaving rat (Bragin et al, 1995). These were seen clearly in the dentate gyrus, where
gamma waves were highly coherent. Average coherence decreased rapidly in the CA3
and CA1 regions. Interestingly, the frequency changes of gamma and theta waves
were positively correlated. Recorded presumptive interneurons in the dentate gyrus
discharged at gamma frequency. These authors suggest that gamma oscillation
emerges from an interaction between intrinsic oscillatory properties of interneurons
and the network properties of the dentate gyrus.

**Theta - Basic features and characteristics**

Theta is characterised as a 5-12 Hz quasi-sinusoidal oscillation in various animals
studied, including rats, cats, dogs, and rabbits. Theta can be associated with alert
attention (eg. in cats) as well as exploratory activity, and REM sleep, but it has been a
matter of debate whether theta can be associated with arousal in rats. In the rat, theta
is always seen in the hippocampus when the animal walks and runs around. Recently, theta oscillations have been seen in humans (Tesche, 1997; Kahana et al, 1999).

Mechanisms of theta generation

Theta generation appears to depend on three factors: 1) cells' intrinsic oscillatory properties; 2) network properties in the hippocampal formation; and 3) external control circuits in the medial septum/diagonal band of Broca complex (MS/DBB) and other areas.

Evidence that hippocampal neurons act as oscillators comes from intracellular slice work showing: 1) that depolarization via injected currents causes membrane potential oscillations in the theta frequency, without need for action potentials, and 2) that the oscillation frequency varies with injected current (Leung and Yim, 1991). Indeed, theta-like rhythms can be generated in hippocampal slices simply by applying carbachol and bicuculline (Konopacki et al, 1992).

Although the network requirement for the generation and in particular the synchronization characteristics of theta across many regions in the hippocampal formation (including entorhinal cortex) is suggested by several studies, the mechanisms are not fully understood (Bland and Colom, 1993).

Evidence for the importance of the MS/DBB comes from experiments in which the MS/DBB is inactivated, and hippocampal theta rhythm is completely lost (Mizumori et al, 1990; Lawson and Bland, 1993). It is likely that both muscarinic and GABAergic projections from the MS/DBB are important. The MS/DBB is not the
only important external region in controlling theta. Many other brain areas are implicated, among the most important of which are the posterior hypothalamus/supramammillary complex and the pons (Bland and Colom, 1993).

Correlates of theta

On the basis of work primarily, but not exclusively, on the rat, Vanderwolf (1969, 1971) reported the following correlates of theta (taken from O'Keefe and Nadel, 1978):

- walking, running straight ahead or backing up, turning, rearing, jumping,
- climbing, struggling when held, swimming, head movements, postural changes,
- manipulation (such as pressing a lever in a Skinner box), and digging in sawdust.

Vanderwolf originally interpreted these correlates as suggestive of voluntary motor activity and contrasted it with the automatic activity correlating with LIA-related behaviours. Later, Vanderwolf played down this distinction. O'Keefe and Nadel reinterpreted these behaviours associated with theta as those which basically involved displacement of the rat. Thus theta in the cognitive map theory was a correlate of moving between places. The next section considers more specific aspects of the relationship between theta and displacement.

Relationship between theta and displacement

An important component of original cognitive map theory (O'Keefe and Nadel, 1978) was that theta frequency might encode information about translation of the animal. In an extensive review of the literature up to 1976, the emergent conclusion from more
than 20 studies was that theta had a high frequency during motor behaviours which involved displacement of the animal (or its head), and a low frequency during those behaviours which did not (most notably lever pressing). Specifically, they suggested that one function of theta “is to shift the focus of excitation within the map from those cells representing the animal’s present position in the environment to those which represent the position it will occupy as a result of movement” (O’Keefe and Nadel, 1978, p179). Whatever the merits of this suggestion, for this to be true, some aspect of theta would have to signal either the velocity of a given movement or the distance which the movement translates the animal through space. What follows is a brief review of some of the work investigating such possible relationships. It is fair to say this is a complex topic, and only a flavour of the work, which even now may be described as preliminary, can be given. It makes sense to begin with Vanderwolf’s claim that while “amplitude of theta is related to the gross amount of concurrent motor activity...frequency increases, on the other hand, are associated with the initiation of movement” (Vanderwolf, 1971, p92).

Whishaw and Vanderwolf (1973) had investigated rat jumping and reported that theta frequency increases at the time of launch. A jump to a height of 22 inches produced higher frequency theta at launch than a jump to only 11 inches. Moreover, Vanderwolf et al (1973, cited O’Keefe and Nadel, 1978) had suggested that placing weights on the rat’s back had no effect on theta frequency. Such findings prompted Morris, Black and O’Keefe (1976) to reinvestigate this ballistic movement. Just as Vanderwolf and colleagues had found, they observed “continuous theta while the rat prepares to jump and...an increase in frequency of theta during the launch” (O’Keefe and Nadel, 1978). Most importantly, they reported a good correlation between the
frequency of theta during the jump and the distance jumped. This correlation was linear at jumps of above 33cm, and where tested, this correlation was unaffected by adding 50g to the animals’ backs.

(Note. There has apparently been a pharmacological study of theta during jumping, suggesting that the pre-jump theta component is atropine-sensitive. I have not been able to find reference for this in the usual databases, going back to the 1960s.)

O’Keefe and Nadel (1978) also reviewed evidence about the relationship between frequency and running speed. This is a somewhat complicated issue. McFarland et al, (1975) for instance had found no difference in frequency associated with different running speeds but rather an increase in theta amplitude with higher speed. O’Keefe and Nadel’s review led them to conclude that “during a continuous movement, such as running on a treadmill or in a running wheel, the initial frequency of theta is a function of the velocity of the moving surface, but with continued movement, the frequency tends to settle down to the same value irrespective of velocity of movement” (p182). Recent work has shown that at least on linear tracks, a positive relationship sometimes exists between velocity and theta frequency (Buzsaki et al, 1982; Recce, 1994). The tuning by velocity is not very great, however, and it is possible that the frequency increase is explained by another factor, velocity being only a secondary correlate. If this is to be investigated further, perhaps making the linear tracks longer will be a good idea.

Czurko et al (1999) examined theta in rats running in a wheel. Similarly to McFarland et al, they found that velocity of wheel running had very little effect on theta
frequency, but that the power at the theta frequency (and at its second harmonics) was larger at faster speeds. Interestingly, they report that the frequency of theta at high running speed was the same as that when the animal stopped momentarily and turned its head or reared in the wheel (though no evidence is given for this assertion). The increased power may relate to their observation that both pyramidal and to some extent theta cells fire at higher frequencies at higher running speeds (discussed further in Chapter 4). It would be interesting to determine what the effect would be of increasing the friction in the wheel. Certainly, at present, Czurko et al’s study (1999) has some consonance with Vanderwolf’s claim that only amplitude and not frequency would be expected to increase with the higher levels of gross motor activity associated with faster running.

2) Cell types, and complex-spike cell firing in the hippocampus

**Complex spike cells and theta cells**

The initial division by Ranck and colleagues of cells in the CA1-3 region has proved very useful (Ranck, 1973; Fox and Ranck, 1975). They recognised two broad groups: complex spike (CS) cells and theta cells. CS cells fired in single spikes or in bursts. Successive spikes in the burst had a tendency to have decreasing amplitude. The firing frequency of CS cells was lower than that of theta cells. Further, the longest burst of a CS cell was typically less than two seconds, while CS cells could be silent for minutes at a time. In contrast, theta cells always fired single spikes, which were of shorter duration than CS cells, and which did not decrease in amplitude. Theta cells could sustain high (up to 150 Hz) continuous firing for many seconds, and increased firing in the presence of the global theta EEG pattern. It has become clear that CS cells are pyramidal cells, and theta cells are interneurons (Fox and Ranck, 1981).
There are several kinds of interneuron, with different morphology, connectivity, and perhaps functional specificity (eg. Buhl et al, 1994). Subsequent work (reviewed in Bland and Colom, 1993) has also identified theta-off cells, cells which reduce firing rate in the presence of theta, as well as the theta-on cells described by Ranck and others.

**The complex spike phenomenon - intracellular and extracellular recording**

If we assume a model where to a first approximation the extracellular waveform is the first derivative with respect to time of the intracellular waveform, then there is a seeming discrepancy between the intracellular slice, and extracellular in vivo, recordings. For instance, in a slice recording study of rat CA1 pyramidal cells, Shao et al (1999) observe of successive spikes in five spike trains “there was little or no change in the spike amplitude or rate of depolarisation” (see eg. Shao et al, 1999). A similar rate of depolarisation on the way to the intracellular spike peak would be equivalent to similar spike peaks in the extracellular recorded waveform. This suggests that the decreasing amplitude of the spikes in the extracellular waveform (Ranck, 1973) is due to the contribution of various extracellular fluid currents to the extracellular waveform in addition to the trans-channel current going into the cell in question. A recent study explicitly comparing intracellular and extracellular recordings of pyramidal cells (Henze et al, 2000) has shown that it is the later part of the extracellular waveform that is most different from the derivative of the intracellular waveform.
**Spike broadening and calcium entry**

Broadening of action potentials during repetitive firing is widespread in somata, dendrites, and nerve terminals in different neural regions in different species, and is well established in rodent CA region pyramidal cells (many references cited in Shao et al, 1999). For instance, in intracellular somatic recording from rodent slices where the amount of current injected is adjusted so as to produce a burst of five action potentials, the spikes after the first spike are typically of longer duration than the first spike, the 3rd is longer than the 2nd, and the 3rd or 4th spike is the longest of all the spikes (eg Giese et al, 1998; Shao et al, 1999). This spike broadening is frequency dependent, such that at higher firing frequencies the broadening increases. It is likely that at least two types of potassium channel (BK and A) are involved in this broadening (Giese et al, 1998; Shao et al, 1999). Since most of the influx of Ca$^{2+}$ ions occurs during the later part of the action potential, where the broadening occurs, since Ca$^{2+}$ ions are known to have roles in plasticity, and since the broadening is frequency dependent, spike broadening in repetitive firing may be a mechanism of increasing the gain of a Ca$^{2+}$-dependent learning signal. Interestingly, knockout mice which lack an A-type potassium channel (Kv1.1) and exhibit reduced frequency-dependent spike broadening, but normal LTP, show a deficit on a version of the hidden-platform water maze task, where the animals have to learn to go to a new location and ignore the previous location (Giese et al, 1998).

3) Long term potentiation (LTP)

It has long been thought, since Cajal's early anatomical work, that the location of memory storage might reside in synapses. Hebb (1949) and Konorski (1948) specifically proposed a coincidence-detection rule in which a synapse made by two
linking cells would be strengthened if the cells in question were simultaneously active.

Bliss and colleagues (Bliss and Lomo, 1973; Bliss and Gardner-Medwin, 1973) described precisely such an effect in the excitatory connections between perforant path axons and dentate granule hippocampal cells. Brief trains of high-frequency stimulation to these pathways caused an abrupt and sustained increase in the efficiency of synaptic transmission. This phenomenon has come to be called long-term potentiation (LTP). LTP has subsequently been found in other regions, such as in the CA3-CA1 synapses, and in neocortex.

As understood by Bliss and Collingridge (1993), LTP refers to that potentiation which lasts longer than an hour and is NMDA-receptor dependent. It may briefly be said that there are other kinds of long-term NMDA-dependent potentiation, which are not LTP in the sense normally understood. These include non-Hebbian LTP, a potentiation which appears not be fully input-specific (Bonhoeffer et al, 1989). LTP can last for up to weeks if the animal is unaesthetised at the time of induction (Bliss and Gardner-Medwin, 1973; Jeffery et al, 1990; Racine et al, 1983).

It is well-established that LTP induction is Ca$^{2+}$-dependent. The precise contribution of extracellular Ca$^{2+}$ influx and intracellular Ca$^{2+}$ store release may not be fully understood, however. Bliss and Collingridge’s review summarised the available evidence up to 1993 as suggesting that “under normal conditions Ca$^{2+}$ permeates NMDA channels to provide a transient signal which is necessary for the induction of
LTP. It is probable that this signal is restricted to the vicinity of activated spines and is amplified by release from intracellular stores” (Bliss and Collingridge, 1993).

Bliss and Collingridge tentatively subdivided LTP into three phases: the first blocked by kinase inhibitors, but not by protein synthesis inhibitors, the second blocked by translational inhibitors but independent of gene expression, the third, with a time constant of several days, only obtained in unanaesthetized animals, and requiring gene expression (Jeffery et al, 1990). Further work appears to have confirmed the separation of an early and late phase; the requirements for a very long phase have not been fully explored. Kandel and collaborators, working on CA1 in hippocampal slices have recognised two phases, early and late, and suggested that late LTP is blocked by inhibitors of protein synthesis or PKA (Frey et al, 1993), and that there is a critical period of transcription for induction of the late phase (Nguyen et al, 1994). This latter study examined LTP to 4 and half hours after induction.

Relationship between LTP and learning and memory - behavioral links

This is a vast topic, outlined with brevity here. The following focuses on hippocampal LTP and spatial memory. Most studies aiming to demonstrate the LTP-spatial learning link can be categorised as falling within three approaches:

1) Tetanising synapses as much as possible so that presumed memory storage has virtually no capacity, and showing spatial memory impairments.

2) Observing correlations between degrees of LTP-abnormality in animals and behaviour in spatial memory tasks.

3) Unit recording of place cells, showing correlations between LTP-abnormalities and abnormal place cell firing patterns.
The tetanization approach has had mixed results, perhaps because of technical difficulties. The first study of this kind (Castro et al, 1989) was not replicated by subsequent studies (Jeffery and Morris, 1993; Korol et al, 1993; McNamara et al, 1993; Sutherland et al, 1993; reviewed in Bliss and Richter-Levin, 1993). A common explanation for the findings has been that it is too difficult to obtain full saturation of the synapses. More recently, an approach which has combined lesions of the hippocampus on one side, with repeated tetanization of the hippocampus on the other side, has fared better (Moser et al, 1998). Residual LTP was studied in this experiment. The authors found that spatial learning as measured by the water maze task was disrupted in animals with little or no residual LTP, but not in animals whose synapses were capable of further potentiation.

Correlations between LTP levels and spatial memory performance have shown mixed results. When genes non-redundantly involved in regulating LTP are deleted, mutant mice can no longer sustain normal LTP, and usually, but by no means always, show defects in spatial learning (reviewed in Elgersma and Silva, 1999; Lipp and Wolfer, 1998). In part, some of the problem here is that there is a great deal that is not fully understood between the manipulations used to affect LTP levels (eg. NMDA-receptor manipulation) and behaviour. There is quite a leap in the level of variables studied, eg. from receptor gene to escape latency in a water maze. Also, this kind of approach is not so suggestive of causal relationships as the tetanization approach. For instance, one recent study (Migaud et al, 1998) created mice with mutant postsynaptic density-95 protein, and showed that these mice had enhanced LTP in all frequency ranges. It is easy to agree with Tsien (2000) that this was an exciting study, showing that the
mice had impaired spatial learning, but perhaps it would have been almost as interesting had the result been that the mice had improved spatial learning. Another recent study (Tang et al, 1999) overexpressed the NMDA receptor 2B in forebrains of transgenic mice, and found enhanced LTP in these animals, and enhanced performance in several tasks including the water maze.

Finally, studies have used genetic manipulation, combined with actual recording of place cells. This kind of study has the advantage of not making such a big leap from receptor to observed behaviour. Observations have not been entirely consistent, but there does seem to be agreement that LTP correlates with stability of place cell representation (eg. Cho et al, 1998; McHugh et al, 1996, Rotenberg et al, 1996).

In summary, although there is much to do in terms of understanding the links between LTP and memory, partly because much is unknown about the relationships between neuronal firing, representation, and observed behavioural variables, the idea that an LTP-like process is involved in spatial learning is certainly promising.
CHAPTER 4

PHYSIOLOGICAL CORRELATES:

REVIEW OF PLACE CELLS, AND HEAD DIRECTION CELLS

The bulk of this background chapter is concerned with the firing properties of the hippocampal pyramidal cells, or place cells. Of course, some investigators do not view the function of the rodent hippocampus in solely spatial terms. It is simpler in reviewing the literature to comment on hippocampal cells in terms of place cell properties. Later sections consider work by some authors reporting apparently non-spatial properties of hippocampal complex spike cells. After summarising much of the work on hippocampal cells, the chapter looks at firing properties of the head-direction cells found in the presubiculum (and other areas).

A) PLACE CELL REVIEW

Locational firing of pyramidal cells (place cells and place fields)

The most basic observation is that complex spike cells, which have been shown to be the pyramidal cells in the CA3 and CA1 fields, fire at relatively high rates in a specific portion of an environment, and relatively low rates outside this portion of the environment. The specific portion of the environment where a given cell fires at relatively high rates can be termed the place field of the cell, which is itself termed a place cell (O'Keefe, 1976).
Basic Place field characteristics

Spatial measurement paradigms and statistics have not developed sufficiently to
describe place fields quantitatively to the satisfaction of all investigators. Some
general properties however can be noted. It is clear that the distribution of firing
within place fields approaches a 2-dimensional gaussian distribution (Breese et al, 89;
Muller et al, 87; Muller et al, 1994; O'Keefe and Burgess, 1996), with peak firing
describing a centre in the field, and lower firing describing its edges. Figure 4 of
Breese et al (1989) shows firing density decreasing in a Gaussian fashion as
normalised distance to the border of fields increases. The shape and orientation of
place fields appears to be a function of the environment the fields are found in. For
instance, crescent shaped fields may often be found in cylinders but are rare in square
and rectangular boxes, where the long axis of elongated fields is parallel to box walls
(Muller et al, 87 esp. fig. 6 and p.1943; O'Keefe and Burgess, 1996; Hartley et al,
2000; this thesis' dataset). The range of peak firing (in spikes of all kinds per second)
assigned to CS cells clearly depends on parameters adopted to describe the place field
and its peak (eg., most obviously, spatial bin size, smoothing levels) and on the degree
of recording time allotted to given cells, but various methods of describing peak firing
can show place fields with up to 30 Hz peaks. The firing rate outside the field, as is
often seen with well isolated cells, can approach 0 Hz.

Multiple place fields of single cells

A single CS cell may fire, with high spatial specificity, in more than one region of a
bounded environment. While it was previously thought that this could be an artefact
of the available recording technology’s inability to distinguish two or more CS signal
sources, and while the quantitative extent of multiple peaks may not be agreed upon
the existence of the phenomenon itself is not in question (eg. O'Keefe and Burgess, 1996).

**Place fields and directionality**

Early reports often emphasised direction-specificity as well as location-specificity for place cells (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976; McNaughton et al, 1983). A place field may be described as directional if the cell fires only, or at a higher rate, during traversals of the field in a particular range of directions. For instance, during radial maze tasks, a cell might fire when the rat runs through a field on outward journeys only. Subsequent studies have addressed this issue and produced the following consensus: in open field environments, place fields are usually not directional, but in environments or tasks where rat traversals are spatially restricted, by environmental constraints (eg. maze arms) or by reward-shaping, fields are often directional (eg. Markus et al, 1995; Muller et al, 1987; Muller et al, 1994; O'Keefe and Recce, 1993; O'Keefe and Burgess, 1996). Many tasks combine both types of traversal restriction. In the 8 arm radial maze for instance, the arms are usually narrow, and an efficient approach to reward-consumption in the task is arguably inconsistent with loitering in parts of arms in all directions. The study by Markus et al (1995) suggests that the directionality seen in place fields is primarily attributable to the rat's restricted behaviour, and only secondarily, where it exists, to environmental constraints. Markus et al reshaped rats who had originally been trained to traverse an open cylinder in a pseudo-random way, to traverse the cylinder in a stereotyped way to obtain reward at four fixed reward sites. The proportion of directional fields grew from less than 20% to nearly 40%.
Place fields and angular location

As discussed in the section on head direction cells, it is likely that the presubiculum direction system exerts control over the angular location of place fields. In some studies investigators take pains to exert experimental cue control over static background and other uncontrolled cues. Rats are shaped to use the experimentally-chosen cues/systems (external or ideothetic) to control their sense of orientation, and conflict between different cues or sensory systems contributing to orientation sense is minimised. In such circumstances, when the experimentally-chosen cues are rotated by a given amount, place fields can be observed to rotate both in unison and by a similar amount to the controlled cues (Jeffery et al, 1997; Jeffery and O'Keefe, 1999; Muller et al, 87; O'Keefe and Conway, 1978; O'Keefe and Speakman, 1987; Olton et al, 1978). This is a well established finding, but it does not imply that the relationship between the angular locations of place fields is always preserved following rotations. Certainly, in situations in which cues and sensory systems are in conflict (eg. visual vs vestibular systems, single cue vs another single cue), a group of individual place fields can rotate by dissimilar amounts around a centre. This has been seen in the dataset of the thesis (not shown), but is complicated by the fact that the experiment was done in a square. A study relating to this issue was recently done by Fenton and Muller (2000). Having first established that 2 cue cards on the walls of a standard cylindrical environment controlled the orientation of the place fields in the cylinder, they altered the distance between these cards. Although this procedure causes translation as well as rotation of place field centres, the result emerges that place fields proximal to the cards are rotated more than place fields distal to the cards.
A key problem for all of these studies is that it is not clear to what extent the hippocampal unit firing is driven by, or independent of, the directional system. Few studies have attempted to find dissociations between, for instance, simultaneously recorded head direction cells in presubiculum and CA1 place cells.

The multimodal sensory nature of the place cell phenomenon

An element of the hippocampal cognitive map theory (O'Keefe and Nadel, 1978) was that place field activity could be derived on the basis of various sensory modalities. In principle, input pertinent to spatial mapping such as distance and orientation information could be obtained and integrated on the basis of several external (e.g., visual, somatosensory, olfactory, auditory) and internal cue responsive systems (e.g., self-motion, proprioceptive, vestibular). Can place field firing be established and maintained on the basis of distinct and/or multiple additive sensory input? What mechanisms are involved? Are some sensory systems privileged in hippocampal place field firing?

Early work by O'Keefe and others (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976; O'Keefe and Conway, 1978) suggested that place units were opportunistic with regard to sensory input. One of the problems with these studies, for our purposes here of showing distinct and additive sensory control, is that relevant information might be extracted from any one or more sensory system’s responses to a “single” cue, like a buzzer or a running fan (visual, somatosensory, auditory, and olfactory). Evidence from the probe trials reported in the O'Keefe and Conway (1978) study, where cues shown to have stimulus control were systematically removed, suggested that visual cues might have had more valency for 3 of 8 cells studied in this way. One clear
observation nevertheless was that a high proportion of place fields could be maintained in the same position in the environment in the dark, at least on the second occasion that lights were switched off (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976). Recent work has further confirmed this (Quirk et al 1990; Markus et al, 1994), though the basic observation is clouded by issues relating to the rat’s previous experience prior to dark trials (Quirk et al, 1990) - as is anticipated by O'Keefe’s observation that some place fields returned only in the second dark condition.

These studies are generally not able to elucidate mechanistic processes of place field firing. One problem with the probe trial approach is that an input shown to support maintenance of a place field is not necessarily an input which singly or in combination with other inputs established the place field initially. Conversely, it is hard to show definitively that place field maintenance following cue removal implies a switch from one modality set to another, since it is often unclear which sensory modality or combination was responsible for the initial establishment. A place cell which continues to fire in the dark on a second trial might be doing so on the basis of self-motion information, a modality which may or may not have helped establish the field. There is not sufficient space to tease out these matters fully here. It is more appropriate to review another line of evidence about multimodality which comes from studies where certain sensory modalities are purposely impaired by experimenters.

Hill and others (1979; reviewed in O'Keefe 1979) attempted to eliminate entire sensory modalities from rats undergoing place cell testing. Animals were blinded or deafened or had their vibrissae removed or had their olfactory receptors destroyed. Place cells were found in all these animals, with no obvious differences from those
found in normal rats. Hill and Best (1981) deprived animals of both vision and hearing: again, place cells were found in these animals. Recently, using quantitative approaches developed by Muller et al (1987) and Skaggs et al (1993), Save et al (1998) compared place cell firing in rats which were surgically blinded one week after birth with that of normal rats. A cylinder, with four different objects placed at the periphery, served as the testing environment. No statistically significant differences were found except that the fields of blind rats were slightly more directional, and that blind rats’ place cells had lower firing rates. The size of the blind rats’ place fields, and their locational informational content were similar (ie. respectively smaller and higher, but not significantly so.) Intriguingly, unlike place cells in normal rats “which fired from the first moment of entry into the environment” (ie. at the start of a given trial, but after previous training in that environment), place cells in blind rats did not fire until the rats had “made physical contact with at least one object”. This might suggest that, for these rats, which might be expected to rely more on ideothetic information, ideothetic information alone was insufficient to reinstantiate place field firing. It might have been very interesting to test firing in a condition where the objects were removed.

The question of which sensory modalities dominate place cells may be misconceived if the goal is to establish some kind of natural prepotency of a given modality in the rat. At any rate, definitive data on this are lacking. The purpose of this section has been to review the evidence leading us to the general conclusion that there is an inherent flexibility and multimodality in the establishment and maintenance of place fields. This evidence is consistent with O’Keefe and Nadel’s view that the hippocampus has been highly determined, by evolutionary processes, to construct
allocentric place fields. Detailed knowledge about mechanisms of sensory integration must await further research.

The relative importance of distal and proximal cues
Hebb (1949) reviewed evidence to suggest that the position habits of mammals with lower processing capacity, such as the rodent, were set up more by distal than proximal cues. From some of the studies examining the angular location of place fields mentioned above, in which it is generally observed that distal cues exert control over local cues such as box odours and maze arm textures (e.g. Jeffery et al, 1997; O'Keefe and Conway, 1978; O'Keefe and Speakman, 1987; Olton et al, 1978), this suggestion appears very reasonable in the context of place field study.

Two recent studies have explicitly addressed these issues, though it should be noted that once again the primary variable under investigation was the angular location of place fields (Cressant et al, 1997; 1999). Cressant et al (1997; 1999) placed sets of objects (a wooden cone, plastic cylinder, and red wine bottle) in the Muller cylinder and found that, when centrally placed, the objects failed to control the angular location of place fields, while the same objects placed at the periphery of the cylinder exerted strong stimulus control over angular location. (The first of these studies gave very preliminary indications that the stability of place cells was reduced in the centrally-placed objects condition.) The authors interpret these results by endorsing reasonable Hebb-like arguments about the reduced computational load of using distal objects to create a stable reference frame. It can be argued that these studies are really, in effect, investigating cue control of directional system cells, but only indirectly so, through hippocampal place firing. Other issues of stimulus control relating perhaps
more to specifically hippocampal function, such as whether distal cues are more important in *establishing* place fields, and controlling place field *reliability, specificity* and so on, have not been tackled.

While undisputed, these observations may be limited in scope by the specific types of tasks used, by the nature of the proximal and distal cues used, and because proximal cues are not made sufficiently distinctive or salient by the experimenters. For instance, if rats were to be overtrained in conditions where visual distal cues did not consistently predict reward sites, but local textural cues did, different results might be seen. It is perhaps mistaken to overgeneralise data about place field control by distal cues, and from behavioural experiments, into a rule along the lines of “distal cues always dominate over proximal cues (or are prepotent)”.

**Place fields and environmental scaling – size and number**

Ignoring for the moment the “absolute size” of place fields seen in cells in different parts of the hippocampus (discussed below: next section), it can be asked if place fields increase in size in bigger environments. Restricted to this simple question, findings are in accord, the answer being that they do increase in area (Hartley et al, 2000; Muller and Kubie, 1987). Probing further into the issue, however, shows up discrepancies. Muller and Kubie (1987) found, once sampling biases were controlled for, that when a similarly-located place field was observed in two environments of similar shape, but different size, the areas of the two resulting place fields differed less in scale than did the areas of the actual environments. Thus, “firing field area does not scale up equally to the scaling of apparatus size” (Muller, 1996). Hartley et al (2000), analysing the data of O’Keefe and Burgess (1996), suggest a different view,
however. Hartley et al measured the area of the portion of each place field firing at 50% or more of the peak rate. Although the dataset is comparatively small (28 place cells), and the issue was not statistically examined, field areas were well predicted by environmental area. The mean field in the small square was $109\;\text{cm}^2$, and the mean field in the large square, four times the area of the small square, was $385\;\text{cm}^2$. The average of the two means in the rectangles, which were half the area of the large square, and double the area of the small square, was $206\;\text{cm}^2$.

*Place field extent within an environment – are there differences in the dorsal and ventral hippocampus?*

How large are place fields? This is a question that is in need of an agreed calibrative framework. Certainly, current answers depend on the quantitative measures chosen by individual laboratories. In what has become the standard environment for the field, the 51cm high, 76 cm diameter cylinder, Muller et al (1987) found that the average field area for place cells was on average 22% of the 2-dimensional circular floor surface, taking 1 Hz as the cutoff point. They found a correlation between field size in pixels and log(cutoff) was $-0.995$. To give a flavour of the relationship, using 8 Hz as the cutoff point gave an average field size of 4% of the circular floor area. In his recent review of place cells, Muller (1996) reported an average field size of 13% of the apparatus, with a 1 Hz cutoff. The reduction (22 to 13%) may well reflect the use of better recording techniques. The use of an absolute value could allow noise to contribute overmuch to the measure. Moreover, as described below, place cell firing rates show excess variance and are not well understood. It is possible that place field maps autoscaled to peak firing rate are more appropriate for considering place field extent. With this measure, the cutoff point becomes a proportion of maximum firing
rate. Hartley et al (2000) reanalysed the data in O'Keefe and Burgess (1996) in this way, to consider the peak area where firing rate exceeded 50% of the maximum, for a given cell. Expressing Hartley et al’s figures as a proportion of the four rectangular environments, peak areas occupied about 3% of the available surface. It should be noted however that each peak was considered as a separate field area, where a cell had more than one field. It is not clear how much larger the 3% would be if all the peaks form a single cell count towards one total field area for that cell, since each field is autoscaled.

It should be noted that to date experimenters have not used large testing environments. Virtually all place cell experiments have been conducted in environments where the available plane for rat to walk on is no more than 1.5 x 1.5m, at most.

Perhaps more importantly, all these figures are based on cells recorded in the dorsal CA fields of the hippocampus. The septal (anterodorsal) and temporal (lateroventral) poles of the hippocampus differ, however, in connectivity and neurochemistry. Briefly, the septal pole receives more sensory input from the external environment than the temporal pole, while the temporal pole receives more input from the rat’s internal environment than the septal pole. With such differences in mind, Jung et al (1994) recorded from dorsal and ventral CA1 cells, and found significant differences in several measures of spatial coding. Dorsal CA1 cells had more spatial information content per spike, higher spatial information rates, more sparsity, and smaller place fields than ventral CA1 cells. The difference in place field size was marked. On average the place fields of ventral cells were more than 4 times larger in area than
those of dorsal cells. Moreover, of cells recorded during slow-wave sleep, 45% in the dorsal CA1 had place fields, while only 18% in ventral CA1 had place fields. In the same year, however, Poucet et al (1994) reported no differences in the size and shape of fields from dorsal and ventral sites. These authors also reported that ventral cells had a lower firing rate, again in contrast to the (not significant) trend reported by Jung et al (1994). These discrepancies will not be considered further. It should be noted in passing however that all the dorsal/ventral differences reported by Jung et al persist, even when only those cells simultaneously recorded from the two regions in three rats are included in the dataset (i.e. 42 dorsal, and 61 ventral cells). While the exact distribution of these cells is not further reported, and may not be even, this finding inspires at least some confidence in the results.

Place cell firing patterns can be different in different environments

Obviously, this issue is one investigated by the present thesis. This summary is kept very brief here, as much of the literature is reviewed in both the Introductory Overview, and the Discussion (Chapter 10) of the present thesis. Early studies by O'Keefe and colleagues indicated that place cell firing patterns could be different in different environments (O'Keefe and Conway, 1978; O'Keefe, 1979). The firing of cells on a holding platform could not be used to predict the firing of cells on a 3-arm raised maze.

Subsequent work confirmed this result (Kubie and Ranck, 1983; Muller and Kubie, 1987) using a variety of environments. Kubie and Ranck examined firing in three environments in different behavioural contexts, all in the same position in the experimental room. The environments used were an 8-arm radial maze, an operant
chamber which the rats had been trained in (a DRL-16 task), and a large home box containing the female rats’ pups. These authors found that complex spike cell firing was indeed related to place in all three environment/behaviour situations, but, like O’Keefe, could find “no relation between the places in each of the situations” (Kubie and Ranck, 1983). Muller and Kubie (1987) adopted a different approach, developing a pellet-chasing task to test animals’ place cell responses in rectangular-walled and circular-walled environments. With similar behaviour in these environments, placed in the same position within the laboratory, these authors found, again, that fields in the rectangle did not predict fields in the circle, and many cells firing in one environment were virtually silent in another. This basic finding has become known as “remapping” (Bostock et al, 1991; Muller, 1996).

As the present thesis demonstrates some limits on differential firing, at least on initial exposures, it seems appropriate to make a statement of the form “place cell firing patterns can be different in different environments.” Such a qualified statement is unquestionably true, and already interesting, since it suggests a neural representation that is not like that seen in much of the neocortex. Further discussion of the “remapping” phenomenon is deferred to the Discussion (Chapter 10).

**Variance in spatial firing**

While place cell discharge is usually well confined to a specific portion of the environment, this firing is not very reliable in the temporal domain. Thus it is easily observed, for instance, that a rat may take a path through a place field in similar ways on two occasions, and on the first occasion the cell may fire several spikes, and on the second fire none. Another way of expressing the phenomenon is to say that $x, y$
position as a single variable accounts for remarkably little of the variance of place field firing. Firing characteristically occurs in the same place, over and over again, but the temporal firing pattern is not reliable. Fenton and Muller (1998) formally analysed this characteristic of place cells in some detail. They compared the firing during rats' passes through place fields with a model using Poisson variance of the location-specific firing as determined by time-averaged positional firing rate distribution. They found that firing was characteristically higher or lower than the model would predict, and called this "excess firing variance". Several factors that could contribute to this excess variance were ruled out, such as speed, theta modulation, and complex spiking.

Robustness in spatial firing – a population code for place

It is clear that there is both robustness and excess variance in place field firing. One measure of the robustness of the code is to determine how accurately the rat's position in an environment can be reconstructed based on reading the spike code of a population of its place cells. Wilson and McNaughton (1993) estimated, by extrapolating the cell number/reconstruction error plots derived from a template matching reconstruction method, that in a box about 1.3 m long and 0.6 m wide, 1 cm accuracy over a 1 second integration time could be obtained from about 130 cells. Fenton and Muller (1998) reanalysed this data, to give similar results, while showing that much of the error in reconstruction is attributable to excess firing. The issue of reconstructing position based on taking information from cell firing has been very interestingly explored by Zhang et al (1998). Briefly, inter alia, they survey many reconstruction methods and show that Bayesian probabilistic methods can be used to reconstruct the position, over a 1 second integration time, of rats running on simple rectangular tracks with an accuracy that approaches (within a factor of two) the
information theoretic limit on how accurate any reconstruction method can be, and that is anyway not very different from the intrinsic experimental errors, according to the authors, involved in their position tracking. (Zhang et al’s use of the 5 cm figure of Wilson and McNaughton (1993) based on open field exploration, is perhaps somewhat generous, since their rats ran unidirectionally on rectangular linear tracks).

There are many issues raised by these studies which deserve further consideration, such as the parameters used (the considerable increase in error at shorter time intervals such as 0.1 second, or approximately 1 theta cycle), the increase in error at low speeds, the unimportance of place field size, and the possibility that the rodent neural system would not necessarily have evolved the most optimal “reconstruction” solution, but merely one that confers fitness. The point emphasised here is simply that three studies have used reconstruction methods from at least two different datasets and demonstrated that the spatial information in place field data is, in principle, sufficiently accurate and robust to serve the organism as a means of determining its allocentric position, despite the fact that position as a single variable accounts for little of the variance in the firing rate of a single place cell.

Robustness in spatial firing – persistence and stability over time

How long do place fields last? The classic answer to this simple question is usually to refer to the observations in Muller et al (1987) and Best and Thompson (1989) that place fields can be stable for as long as the recording electrodes are presumed to be stable with respect to a given cell. Thus Muller et al found some cells with stable place fields over 6 days, as did Best and Thompson (n = 9) over 5 days. The longest stable field to date was recorded by Best and Thompson in a six arm radial maze over
a 153 day period. (This thesis presents data from several cells with stable fields in at least one environment beyond a 2 week period.)

There are at least three complications, however, related to learning and “remapping” (and which bear directly upon the present thesis). Firstly, neither of these studies, nor others to my knowledge which address the question of long-term stability, record from the cells from the first moment of entry into the environment. Thus the stable fields of Muller et al (1987) and Best and Thompson (1989) are seen after a period of training. The standard method of Muller and colleagues (eg Muller et al, 1987; Muller and Kubie, 1987; Quirk et al, 1992) is to overtrain the rats in environments and then implant the electrodes. “The purpose of overtraining is to minimize the likelihood that the first cell or cells recorded from a given animal will systematically differ from other cells because the animal is in an active state of learning” (Muller et al, 1987). At present, then, we only know that place fields can be stable after some period of training in the environment. It is possible that place fields formed on initial exposure to the environment may not be so stable. (The dataset of this thesis suggests that a significant proportion can persist for long periods, well beyond a week, where this can be determined. This thesis does not directly present the results for this claim.)

The second complication is that repeated exposure to several environments may alter the place field stability (the representation or map) in any one or more of the environments. For instance, stable place fields may have been recorded from one environment after training in the usual way, and found to be stable. Then, exposure to other environments might alter the place field stability in the original environment. The 153 day place field stability found by Best and Thompson may be related to the
fact that their animals were not continually exposed to several different environments.

It is sometimes assumed that “when the rat is familiar with [more than one environment] the maps are in the steady state” (Bostock et al, 1991; my emphasis). While it is unclear what degree of experience is sufficient for “familiarity”, this remains an unproven assumption.

Finally, place fields in a given environment can in some circumstances be altered by reshaping the behaviour that the rat executes in that environment (Markus et al, 1995). While it is clearly inappropriate to refer to this phenomenon as a kind of temporal instability (eg. the place field can return to its original location when the rat returns to the previously shaped behaviour), it does circumscribe some limits to what might be termed the “persistence” of the place field.

In summary, it is established that the place fields can persist stably over weeks and months, but the range of circumstances within which this stability occurs is not well characterised.

The phase shift phenomenon

O’Keefe and Recce (1993) observed an interesting relationship between the theta wave of the EEG and place field firing, in rats running on a linear track. They found that as the rat arrived in the place field, spikes fired late in the theta cycle, and as the rat left the field, spikes fired early in the cycle. A phase shift or phase precession occurs, such that each progressive spike burst tends to fire earlier and earlier in the cycle. This was seen in CA1. The authors suggested that there might be an additional neural code at work here beyond rate coding. For gaussian place fields, rates do not
distinguish between various portions of the edges of a field. Phase code detectors however could in principle determine the rat’s position more precisely, giving some idea of which portion of the edge of the field the rat is occupying. This at least is true of what are in effect one-dimensional fields. In two dimensional environments, such as a rectangular floor, directional information would also needed to decode the x, y position more precisely.

Burgess et al (1994) and Skaggs et al (1996) have made preliminary investigations looking for phase shift in open field foraging. It appears that phase shift is less robust in the open field, but can be seen in some cells. It is possible that technical and analytical progress will be required before the open field effect is seen fully. Skaggs et al (1996) did find in the 2 dimensional environment the clear result that spatial information content and spatial specificity were increased in the early portion of the theta cycle. This effect was less clear in the linear environment.

Skaggs et al (1996) replicated the basic phenomenon of phase precession on a linear triangular track, for both CA1 and dentate cells. The phenomenon is less pronounced with dentate cells. This suggests that CA1 may at least partly inherit its phase precession effect from earlier processing stages. O'Keefe and Recce had suggested that the effect might be produced in CA1 due to the frequency of information impinging on the CA1 cells being somewhat higher than the cells’ intrinsic oscillatory frequency. While this mechanistic explanation may be correct, it should perhaps now be placed at an earlier stage of hippocampal processing. There is no current data on phase shift in CA3 cells.
Speed-related increases of place cell firing

McNaughton et al (1983), Wiener et al (1989), Zhang et al (1998) have found that for many place cells, firing rate usually increases in the place field when the rat runs faster through the field. This observation has come from different paradigms. For instance, McNaughton et al (1983) tested rats running a radial 8 arm maze, and found that both CS and theta cells increased discharge with higher velocities, but “showed only a relatively small increase over the range of velocities observed”, and the relationship tended to asymptote at higher velocities, especially for theta cells. The distance from the maze centre to the arm end was 67.5 cm, and it can be argued this is insufficient for the rat to display a wide range of speeds, and to examine the effect of speed changes without the influence of other variables. This problem is more obvious with Wiener et al’s study (1989), where the testing environment was only 40 by 44 cm, and the type of task used gives some parts of the environment more salience than others. Nevertheless, of 293 place fields evaluated, 47% had significant speed tuning, and of these only 7% fired maximally at slower speeds, while 93% showed some relationship where increased firing was seen with higher speeds. As with McNaughton et al (1983), more or less linear relationships sometimes broke down at higher speeds. The data component of Zhang et al’s study (1988) was taken from 2 rats running on rectangular linear tracks (one rat on a figure-8, the other on a simple 4 side, track). Only averaged data, across all place cells, was reported for each animal. The two plots thus produced show a very tight linear relationship (including at the highest speeds) between speed and firing rate ($r = 0.991$ and $0.992$ for the 2 animals). It is not surprising that the best relationship between speed and firing rate was seen in this study, with rats running repetitively in a single direction on tracks.
Such studies have generally tried to examine speed/firing frequency relationships while exploring the influence of several variables at a time. A recent intriguing study by Czurko et al. (1999) attempted to completely circumvent this problem by "space clamping". In this paradigm, rats are trained to run in a running wheel set in a fixed position within a box. The rat's head in the wheel will then occupy, by happenstance, at least some portion of the place fields of cells which are active in the box in that location. They described the properties of 12 cells which were selectively active in the running wheel. As a whole, these cells were strongly modulated by the direction of running. In the preferred direction, a clear positive linear relationship between running speed and firing frequency was seen for all 12 cells. In line with the earlier work described above, the linear relationship was broken at higher speeds (higher than those tested by Zhang et al, 1998), such that firing did not increase beyond some speeds, and even decreased in some cases (cf. Figure 6; Wiener et al, 1989). In contrast to the linear relationship between speed and cell firing frequency, the speed of wheel running had very little effect on the frequency of the theta wave.

One of the problems with this study is that although the degree of "space clamping" is certainly high, it is not necessarily complete. As the animals run very fast, their heads may not be in the same position as when they run slowly.

As a whole, these studies suggest a positive relationship between place cell firing rate and speed. It appears that the correlation is best seen in a given direction, with unidirectional running.
"Anticipatory firing" of place cells

Muller and Kubie (1989) opened up an interesting line of inquiry into locational and other spatial coding by timeshift analysis. In this kind of analysis, the time series describing the occurrence of spikes is shifted relative to the time series describing the location (or other observed variable) of the rat. The idea is then to observe the timeshift which most optimizes certain measures of spatial coding, such as, for place cells, spatial coherence, field size, information content and so on.

The basic results themselves have not been entirely convergent. Muller and Kubie (1989) found that spikes must precede the rat's position by about 120 msec to optimise 3 measures of place field specificity, while Sharp (1999b) found that spikes should precede the rat's position, at average running speeds, by about 30-40 msec to optimise 4 measures (3 of which were very similar to Muller and Kubie's). It is encouraging that the 3 measures of Muller and Kubie gave very similar results (Firing area = 114 msec, Patchiness = 119 msec, and Coherence = 124 msec). It must be mentioned that Breese et al (1989) examined this issue, inter alia, in a study aimed at detecting place field changes resulting from altered reward contingencies. Unfortunately, presumably because these authors did not find a timeshift effect, they did not supply any detailed information of measures used and results obtained, to buttress their report that past and future timeshifts had nearly symmetric effects. For at least three measures of average field size (one of which is probably similar to Muller and Kubie's) they found that smallest fields were associated with a zero timeshift. One difficulty with their report is that the rats' behaviour in their task is not spatially homogenous, but focused on reward locations, where the rats were also, one
suspects, sometimes stationary. Their task may thus not constitute a fair test of a movement-related hypothesis.

However, even if we ignore this result for argument's sake, and summarise Muller, Kubie and Sharp's findings as an agreement on the interpretation that, in Muller and Kubie's words, place cell firing predicts the future position of freely moving rats, significant problems still remain.

The most important of these, is that, somewhat surprisingly, we do not know (in any of the three studies mentioned) where the LEDs tracked by the camera were positioned on the animals. Both Muller and Kubie (1989) and Sharp (1999b) appear to have reanalysed mostly old data for their analysis. Muller and Kubie seem to imply in their discussion that LED placements above the ears might have been common, but this is far from clear. It is premature to describe the firing of place cells as predictive of future location, until studies tend very carefully in advance to LED position. (A further confound is that a rat is likely to spend most of its time in the Muller foraging task with its head bent down to the floor, making the LED positions more anterior than when his head is horizontal.) It may for instance be more appropriate to interpret such data as implying that the rat's spatio-cognitive centre is at a midpoint just ahead of its eyes. It should be emphasised that this is a related but separate issue to anticipatory firing, as Muller and Kubie acknowledge. This speculation generates subtly different, but entirely testable, predictions to the interpretation advanced by Muller and Kubie. A further complication is running speed. In Sharp's (1999b) dataset, the optimisations implied that place cells predicted locations at slow speeds, but lagged behind locations at high speeds. Interestingly, Sharp notes that the larger
"anticipatory firing" interval of Muller and Kubie (120 msec) could be explained by the fact that their rats had slower running speeds than Sharp’s. Lastly, as reported by both studies, there is a great deal of variance in the population: one cell might on average predict location by 300 msec, another may lag location by 200 msec.

This line of analysis, then, while likely to generate fruitful research, must be considered as preliminary. Among the most promising indications are the non-intuitive results that, on average, Subicular cells “anticipate” location more than hippocampal cells (Sharp) and that CA1 cells “anticipate” location more than CA3 cells (Muller and Kubie). For the moment, the idea that place cell firing anticipates location has clearly not yet acquired the status of fact.

**Homogeneity of place fields within an environment**

Initially, it was thought that place field characteristics and distribution were homogenous throughout an environment (O’Keefe, 1976; O’Keefe and Speakman, 1987). Figure 4a shows 13 place fields from the current dataset, whose place cells were recorded from one tetrode. Some recent studies have begun to suggest there may be inhomogeneities. With regards to distribution, fields may tend to be preferentially located near the edges of environments (Hetherington and Shapiro, 1997; Muller and Kubie, 1987; see Figure 8c in this thesis), and above-average numbers of fields may be clustered proximally to salient cues (Hetherington and Shapiro, 1997). With regard to characteristics, fields nearer the edges of the environment may be smaller than those near the centre, in both walled environments (see Figure 4a) and open platforms (O’Keefe et al, 1998). These tendencies are not necessarily very pronounced, and deserve further study, with large numbers of cells.
Figure 4a. 13 simultaneously recorded place cells from one tetrode, showing place fields homogenously distributed in the environment.
Topography of place fields within the hippocampal neural structure

Much more controversial are claims for anatomical-topographic relationships. The vast majority of studies have not found any such relationship (examples include Muller and Kubie, 1987; O'Keefe and Nadel, 1978; O'Keefe et al, 1998). Figure 4a shows that a group of 13 cells recorded from one tetrode show no obvious sign of field-clustering in one area of the environment.

It is perhaps of interest that those studies which have made claims for such relationships (Eichenbaum et al, 1989; Hampson et al, 1999) have involved tasks beyond the simple food-chasing paradigm. In these tasks, behaviour is not expected to be homogenous throughout the environment, and salience is not evenly distributed. Recently, Hampson et al (1999) have claimed that in a delayed non-match-to-sample task, cells associated with position (binary: either “left” or “right”) are arranged in anterior-posterior segments. This might well imply network self-organisation as the task progresses. There are some technical problems with this study, and it will be interesting to see if it can be replicated.

Differences between CA3 and CA1 cells: firing rates, field size, directionality, spatial specificity

Various studies conflict on this issue. Olton et al (1978) and Muller et al (1987) found that place fields of CA3 cells were indistinguishable from those in CA1. In the latter study, the average firing field area (as a proportion of the environment) for CA1 cells was 22.3%, for CA3/4 cells 22.0%; the average maximum firing rate was 15.1 Hz for
CA1 cells, and 20.9 Hz for CA3 cells, the difference falling just short of 0.05 level of probability (Muller et al, 1987).

McNaughton et al (1983) reported that the “place/direction specificity of CS cells was significantly higher in CA1 than in CA3”, yet two of the same authors concluded in 1990 that CA3 place fields had higher spatial specificity (Barnes et al, 1990). Markus et al (1995) reported that CA1 cells had larger fields, and slightly lower spatial information content, than cells recorded in the CA3/dentate region. (Unfortunately this study was not able to differentiate between cells recorded from the dentate and CA3). It might be said that the differences, though statistically significant (both measures, p < 0.01), were not very great. These authors found that CA1 cells had a much higher mean rate of firing than CA3/dentate cells, however, which may be at odds with Muller et al’s observation of higher maximum firing rates for CA3/4 cells, though no in-field firing rate measure was given. In contrast to McNaughton et al (1983), no differences were seen in directionality of CA3/dentate and CA1 place fields.

It may be suspected that techniques are not sufficiently developed to reach consensus. Certainly, this may be true of data analysis. Many issues are beyond the scope of this summary, but it is worth noting that CA1/3 differences are often derived without taking into account effects of experience. For instance, it will often be the case that CA3 cells were recorded in an experiment after CA1 cells, to get more data from the same electrodes. Moreover, cells are usually pooled together for analysis, regardless of the individual rat they were recorded from, and despite the often very uneven distribution of cells among regions and rats. As well as differences in the rats’
behaviour (with respect to evenness of coverage, running speed etc.), there is also the possibility that such parameters as LED positions subtly differ with each rat.

**Place field establishment**

As hinted above (section "Robustness in spatial firing, persistence over time"), the dynamics of place field establishment in novel environments have not been studied a great deal. There are good reasons for this. The first arises from the technical difficulty of getting relatively homogeneous coverage of an environment from a rat during its first few minutes of exploring it. The second arises from the more conceptual difficulty of what constitutes a novel environment. Experimenters who have obtained data about the dynamics of firing upon initial exploration have come up against the conceptual problem, presumably precisely because of the technical difficulty. Thus, one might almost argue that if the experimenters have been able to assure themselves that a rat will run well in an environment, this is precisely because they have contrived, somehow, to reduce its novelty. There are only two published studies which explicitly address this topic, and they are not in agreement.

Wilson and McNaughton (1993) made a reasonable compromise with the 2 problems mentioned. They used a rectangular box with a partition dividing the box into equal halves of about 60 cm in all 3 dimensions. One half of the box was blocked off by a partition (area B). 3 rats were trained in the other half (area A) for 10 days. The idea was to test firing in the "novel" environment of area B, and compare it with that of A. The testing day had 4 phases. Phase 1: rats ran in area A for 10 minutes. Phase 2: The partition was lifted, and rats ran for 6 or 10 minutes in area A and B. Phase 3: A
further 6 or 10 minutes in area A and B. Phase 4: The partition was replaced and rats ran for 10 minutes in area A.

2 of the 3 rats showed hesitation in area B, one so much so that it was ignored for data on place field formation. The chief results for our purposes here were as follows. The pixel by pixel spatial firing correlations between phases 2 and 3 in area B (novel) were “low” (no quantitative measures were given), while the correlations between phase 1 and the three successive phases 2, 3, and 4 in area A were high (mean correlation coefficients were all at least 0.62, p < 0.05). More cells had fields in area B in phase 3 than phase 2, while these numbers were always lower than the numbers with fields in area A. The degree of error in reconstructing the spatial trajectory of the two rats in area B was higher in phase 2 than in phase 3.

These data indicate: 1) That place fields formed in 6 to 10 minutes of area B. 2) These fields were not very stable. 3) Stability improved in area B. 4) The continuous area A+B did not destabilise the representation of area A. As quantitative measures were not reported for the spatial firing correlations in area B, and for the reconstruction error in area A and B (except in phase 1 in area A for rat 1), it is impossible to confirm the authors’ claim that the “spatial representations...improve rapidly with experience” (my emphasis, Wilson and McNaughton, 1993). There is only qualitative report to show that it does improve.

Nevertheless, they provide useful data suggesting that the initial formation of place fields are not as stable as place fields in environmental areas that are familiar to the rat. As such, their results do not tally with the somewhat preliminary study with few
cells by Hill (1978), who found that initial fields were indistinguishable from fields formed by the rats another environment.

One might summarise this section by saying a good study of place field formation does not yet exist, and should be a priority for the hippocampal unit recording field.

**Relationship between place field firing and animal spatial behaviour**

This is clearly an underinvestigated relationship, and one that O'Keefe and Speakman's (1987) study set out to address. These authors used a four-arm maze in a cue-controlled paradigm where the angular location of fields was consistently controlled by the orientation of a set of cues. In a critical test where no cues were presented, the experimenters could not predict the angular location of fields in advance. However, knowledge of the location of a given cell’s field could be used to predict which arm the rat considered as its goal. This suggests a tight relationship between the hippocampal system and spatial problem solving. This experiment may be interpreted as providing information about the directional system’s relationship with behaviour, and the relationship between the directional system and the hippocampal cells. There is much less information about the specifically hippocampal cell-behavioural relationships.

One important finding was that of Markus et al (1994). These authors found that it was the reliability of place cells (ie. their field-stability over time) rather than the spatial specificity of fields that correlated with successful performance on the standard, win-shift, radial maze task (8 arms). This is not particularly surprising, but has important implications for the current information-theoretic approach to place cell
firing (Skaggs et al, 1993). Emphasising the smallness of a place field does not capture place cell function especially well. An interesting theoretical study by Zhang et al (1998) suggests that, speaking purely in terms of a code for precise localisation, smaller fields are no better than larger fields, so long as the peak rates are similar.

In summary, current indications suggest that there are causal relationships between place cell firing and overt behaviour.

**Place field persistence under conditions of altered goal and/or reward contingencies**

Do hippocampal place cells encode the location of goals? Do place fields change their location in a predictable manner following alterations in the location of reward? The results of studies examining these questions have been very divergent, and the issue remains controversial. Although many studies such as Wiener et al (1989), and Gothard et al (1996) impinge on the issue of goal encoding by hippocampal CA cells, only those studies designed specifically to examine these questions will be summarised here. In chronological order, these are the studies of Breese et al (1989), Speakman and O'Keefe (1990), Markus et al (1995), and Kobayashi et al (1997). In the baldest terms, pretending initially for the sake of argument that the designs in all these experiments were similar, changing reward location altered place field locations in as few as 2/19 cells (Speakman and O'Keefe, 1990), or as many as 40/47 cells (Breese et al, 1989), with the other studies in between these extremes. Unfortunately, there is no simple way to account for these differences without some resort to describing methodological differences. Before this description, it is useful to ask what differences could be important. 1) If place fields do not alter, was sufficient time given to the rats to develop the place field changes? Is the amount of experience in the
various testing conditions similar? 2) If place fields alter, can it be shown that these were due to the experimenters’ altered conditions? Do the experimenters have a return-to-baseline or somesuch condition to show that the fields return to their original locations when the previous reward contingencies are reinstated? 3) When goals are altered, what other variables are altered that might explain differences? 4) What are the criteria for describing cell firing as exhibiting place fields?

One of these studies (Speakman and O'Keefe, 1990) essentially found evidence against the view that altering goal location alters place field location. In the initial condition, the goal was the outer end of one arm in an elevated plus maze in a cue controlled environment, such that the goal location was moved relative to the room, but was always fixed relative to a constellation of distal cues. Thus place fields were recorded exactly as in O'Keefe and Speakman (1987). In the altered goal condition, the goal was rotated 180 (or sometimes 90) degrees with respect to the constellation of cues. Importantly, recording did not begin until rats (n = 3) had relearned the new goal to a criterion of 9/10 correct trials. One rat underwent a series of altered goal locations, and another experienced three goal locations in all. Thus, it cannot be objected that insufficient experience might account for the failure of the place fields to move with respect to the goal. Since the fields did not move (or only 2/19 did), it was not incumbent on these authors to test a return-to-baseline condition, though gathering their data from more than one altered goal location condition fulfilled a similar function, and did not show any reported inconsistencies with the main result. An important feature of this goal-alteration experiment is that behaviour was roughly similar in each condition. Dwell-time analysis showed that 2 animals had a dwell time
bias towards the goal, irrespective of its location, and one showed a bias towards arms near 3 of the 6 cues, irrespective of the goal location.

The study by Markus et al (1995) compared a pseudo-random search condition where rats ran for food which could appear in any location, with a directed search condition where food would only appear at four fixed locations. This was performed for both an elevated circular platform and an elevated plus maze. In the directed search condition the experimenter tapped a finger at the baited location, and once the rat ate from that location, tapped a finger at the next baited location. The locations were baited sequentially in clockwise or anticlockwise sequences, generating stereotyped running. The basic results were that, from the random to the directed search, 40% of fields changed on the platform, and 20% of fields changed on the plus maze. An additional 2 rats were tested on the platform from random to directed and back to random search. Of those place fields whose location was altered by the directed task, 23/29 (79%) returned to their previous locations, with 3 more showing fields that were a mixture of the two locations. (How many fields were altered for these 2 rats is not reported.) Although the authors report that the changes were abrupt, this is not supported by the figure they use to show this abrupt change, and the dynamics of place field change were not quantified.

Markus et al’s study appears to have been designed to show that different tasks in the same “mazes” can cause place fields to shift, and it is convincing in this respect. What is less clear is what aspects of the task cause the shift, since the behaviour of the rats in the two conditions differs greatly. The very different locomotion patterns (such as unidirectional running, and narrow movement trajectories), as well as the alteration of
reward contingencies, are likely to be important. The fact that only 20% of fields shifted in the plus maze task would be consistent with this view, since the rats' have still to move through all portions of the maze, in a manner forced by the structure of the maze. It is surely the case that behaviour differs less in between the two conditions in the plus maze.

Kobayashi et al (1997) used intracranial lateral hypothalamic stimulation techniques to study place fields in a 150 cm diameter cylinder in three conditions, bearing some similarity to those of Markus et al, and Breese et al (1989). The first condition was a random search. In the second, based on where a place field was found in the random search condition, the rat shuttled between the place field area found in random search, and a second area defined by the experimenters to be as far away as possible from the place field area, and the rats were rewarded only in these two areas. It is important to note that this is not simply a switch from random to directed search as in Markus et al’s study, since the experimenters choose the goal locations on a case by case basis, where one of the two goals is a place field site. Partly because of this, one presumes, Kobayashi et al’s analysis did not appear to include the outcome of fields outside the now-rewarded areas but within the regions of space the rat ran through in both conditions. At any rate, these authors reported no alterations in the place correlates of the cells between these two conditions. This stands in contrast to the study by Markus et al (1995). In the third condition, only one of the two areas rewarded in the second condition was now rewarded. This goal area was not the place field area but the other area furthest away in the cylinder. The rat had still to visit the place field area however, otherwise it would not be rewarded in the goal area. Accordingly, the differences in behaviour produced by the rats between the conditions 2 and 3 were
reduced, though one might infer from the figures that sometimes, and certainly initially, the rats had more ranging search paths in condition 3 than 2. (Other changes in this somewhat complicated paradigm can reasonably, perhaps, be ignored here.) In the simplest terms, it could be said that the goal areas have been reduced from two to one, but this is not quite accurate since the place field area is secondarily rewarded.

The reported result was that 6/31 cells (19%) changed their fields from the 2nd to 3rd condition. The data does not appear to be very robust, however. Two of the 6 changes are represented in figures for two cells. It is notable that both the cells in question fire at low rates, the lowest of all the examples shown, at about 1 Hz in the fields. One example is of a cell that purportedly developed a new, second field. Yet the figure (Fig. 11) clearly shows that there is a suggestion of a field in both the 1st and 2nd condition. The second example (Fig. 12) shows a cell’s gradual changes in the centroid of its field from the 2nd to the 3rd condition. are depicted. If the shifted field is due to the imposition of the third condition, why is it that the biggest changes come during a return to the 2nd condition? A big lacuna in this experiment is the lack of baseline trials, where the 1st condition would be reimposed. For all six cells, it may be said that the changes would have occurred regardless of reward contingencies. One might argue for instance, that the changes reflect instability, especially since the firing rates are so low. If the two cells represented in the figures are the best examples of the phenomenon, then it seems legitimate to doubt the main result.

The study by Breese et al (1989) obtained markedly different results from all the above studies, despite sharing methodological similarities with the Markus et al and Kobayashi et al studies. Rats traversed an elevated, open square platform in which
five water cups were located at its corners and centre. In the random condition, all
cups were pseudo-randomly baited, while in the second condition, only one (or
sometimes two) cup(s) were baited. For some cells (it is not clear how many) the
experimenters altered the location of the particular cup that was selectively baited.
Although it was primarily a descriptive study, they reported that 40 of 47 place cells
demonstrated shifts in place fields, the paradigmatic change being that a field changed
its location to the region of the selectively baited cup. Other changes included shifts
from one selectively baited cup to another selectively baited cup. Naturally, as we
have seen with this type of experimental design, traversal patterns were altered.

Speakman and O'Keefe (1990) suggested that in this relatively cue-impoverished
situation (curtains closed off the room, and a cue card was suspended on one side) that
the sensory aspects of the reward might act as an additional polarizing cue, and thus
that the results might be accounted for by changes in sensory rather than motivational,
incentive, or reward aspects of the selective baiting condition. This is very reasonable,
but seems unlikely to account for as many as 85% of fields shifting. (It is perhaps
worth stating that such an explanation could not account for Kobayashi et al’s results,
if they were to be considered convincing, since they used lateral hypothalamic
stimulation as a reward.)

At least two other explanations are possible. The first springs from Figure 8 of the
Breese et al report. This shows a composite firing rate map from 35 different place
fields. It is striking that the place field distribution is very heavily biased towards,
indeed is almost exactly consonant with, cup locations. With respect to field
distribution, the baseline condition is thus very different from the random search
conditions of Markus et al, and Kobayashi et al. We also find that recording began with little training, “after the animals would explore the apparatus without showing prolonged bouts of inactivity (1-5 acclimatisation sessions)”. No attempt was made to ignore firing during lack of movement in the presumably still-occurring, if less frequent and prolonged, “bouts of inactivity”. All these indications suggest a rather casual attitude to the criteria for determining a place field. It seems very plausible that many of the so-called place fields are the result of firing during ripple and LIA-type EEG states, which are frequently seen during consumption, and relative stasis. If this is the case, then the altered place fields are simply due to ripple and LIA-inducing behaviours in different places. This explanation is given further plausibility by the figures (Figures 9, 10, and 11) showing place field changes: the “fields” almost always occurred where the rat spent most of its time.

Another explanation, which may be alternative or additive, is that some fields “remapped” due to different, stereotyped, traversal patterns, in the manner of Markus et al. This can also apply to the results of Kobayashi et al.

In summary, those studies purporting to show changes as a result of changes in reward contingencies and goal locations have not been well controlled. As we have seen, one of the major differences between Speakman and O’Keefe’s study and the others mentioned here is that Speakman and O’Keefe, quite simply, altered the location of a single goal. While the study of Markus et al (1995) is quite convincing in its main results, its own arguments support the view that it is the altered behaviour in response to altered reward conditions, rather than the altered reward conditions themselves, that produces place field changes. Bearing in mind that Kobayashi et al
apparently failed to replicate the main result of Markus et al, it seems that this altered
behaviour may be necessary but is not sufficient to cause place field alterations. The
most obvious conclusion of this section is that reward-related information is not a
privileged input set to place cells.

B) HIPPOCAMPAL CELLS - NON-SPATIAL CORRELATES?

Chapter 5 will consider the various theories and descriptions of hippocampal function.
Some of these do not conceive hippocampal function in purely spatial terms, though
all theories clearly have to try to account for the features of hippocampal cells
described above. This section considers claims for non-spatial correlates. This is a less
studied area, but one of obvious theoretical importance. It is clear that correlates may
be interpreted in different ways. The following focuses on approach correlates,
mismatch correlates, and sampling correlates.

1) Approach cells

One correlate that was found by Ranck (1973) concerns approach to a goal. The basic
observation here is that cells may fire at increased rates when goals are approached.
Thus Wiener et al, (1989) found that some cells fired maximally as the rat approached
an odor port or a water cup.

It is obvious that such correlates are not inconsistent with spatial views of
hippocampal function, since the ports and cups are in particular places. Increased
firing towards a place can clearly be interpreted in terms of a directional place field.
Remember from the study by Markus et al (1995) that when trajectories become
stereotyped as is very likely in the Wiener et al study, place fields become directional.
2) Mismatch cells

Again this phenomenon was one originally explored by Ranck (1973). In turn O’Keefe (1976) reported “misplace” cells. It is probably fair to say that “misplace” cells have been underexplored by the field as a whole. Broadly two types were reported by O’Keefe. The first may be described as sensitive to behaviour-or-object-in-place, eg. when cells increased firing when a cup expected to be in a place was removed. The second were more global in operation, eg. increasing firing when environmental conditions were changed. It is possible to reinterpret the second category of cells as perhaps those associated with “remapping”. The first kind of response is very interesting, and may be similar to the findings of “non-match” correlates in non-match tasks (eg. Hampson et al, 1999). Again, however, the spatial framework in such responses is very obvious.

3) Cue-Sampling correlates

These have been found, among others, by Wiener et al (1989), and indeed O’Keefe (1976). Here arguments focus on two issues: the consistent spatial framework, but also that sampling correlates may be associated with theta cells, rather than pyramidal cells.

In the study by Wiener et al (1989) no more than 20% of cells had cue-sampling correlates. Of this fifth of the total, only 13% fired differentially during sampling of a particular pair of odors, as opposed to another pair, in the same position. Thus only about 2.6% of the total cells had clear responses to cue-sampling which cannot be explained in terms of a consistent spatial framework.
O'Keefe has argued that insufficient attention has been paid to the possibility that some of these cue-sampling cells might be theta cells.

**Wood et al, 1999**

In order to rebut the criticisms that the interpretation of non-spatial correlates is at least uncertain when the tasks involve behaviours directed at/in certain places, recent efforts have focused on tasks which consistently alter the position of a particular cue. A study by Wood et al (1999) was designed precisely in the light of these concerns. This study focused on pyramidal cells only, as far as could be judged by waveform peak-to-valley width. The task was a successive olfactory discrimination non-match task, in which cups containing odors were placed in different locations. Altogether there were 9 cup locations, and 9 odors.

Anova showed that about 8% of cells had cue-sampling correlates (irrespective of location), 11% had location-specific correlates, 13% had pure match/non-match correlates, 20% of cells had “approach” correlates, and many more cells showed interactions between these variables. O'Keefe has made comments on this study in a review on apparent non-spatial correlates of hippocampal cells (O'Keefe, 1999). The following comments are largely restricted to those not previously made. Chapter 5 contains further discussion of this paper in specific relation to the theory of hippocampal function espoused by Eichenbaum.

In my view, it is not good practice to look at firing only around chosen time-points, as was the basic analysis method in this study; this does not aid understanding,
especially when the task is only run once. Only 108 trials were recorded (much less than 2 (match/non-match) x 9 (locations) x 9 odors). This is additionally important for the reason that hippocampal pyramidal cells have variable firing rates. It may be suspected that these rates can contribute to spurious interpretations, in so much as a low proportion of cells may vary by chance in tune with any variable and conjunction of variables. This problem is exacerbated when the session is long, and criteria for inclusion in the analysis is only 100 spikes in total. In my experience, and that of O’Keefe (personal communication), a cell may may “shut off” after a while, or take a while to “start up”, for reasons that may not relate to the experimental variables in question, as far as can be understood. There is a concern that cells that had very few burst episodes would enter into the analysis.

The other problem with not analysing the data for all times is that a correlate such as “approach” may not be the most appropriate interpretation for a cell. These authors did not use the same statistical analysis as for the other cell characterizations, but simply compared “the firing rate during the 1-s period beginning 3s before the behavioural response, when the animal initiates its approach to the cup, with that during the 1s immediately before the behavioural response”. It is obvious that speed and possibly other correlates could explain such a difference, but speed was not even measured. This is an example of the way in which the study is well designed but inadequately analysed.

One of the simple things to do in such a situation is to do some subsequent testing. For instance, for a cell with a pure odor correlate, test the cell after the task. Does it still show the correlate?
At any rate, it is not surprising that hippocampal-lesioned animals are not impaired in this task (Burton, O'Keefe and others, unpublished data). It will be interesting to see if the basic results of the Wood et al study are replicated.

C) HEAD-DIRECTION CELLS - PRESUBICULAR CELLS

Head direction cells were discovered by Ranck (1984), and have attracted much attention since. The basic property of a head-direction cell (HD cell) is that it fires maximally when the animal’s head is pointing in a given compass direction (Taube et al, 1990a, b). Since their discovery in presubiculum, they have subsequently been found in the anterior thalamus (Taube, 1995), and in regions projecting to the anterior thalamus (Blair and Sharp, 1998). There have been claims for head-direction cells in other areas, but these do not seem to be like the HD cells in anterior thalamus and presubiculum.

The following is a brief listed summary of HD cell properties, and focuses primarily on the presubicular HD cells.

1) An HD cell shows an increased firing rate for a particular compass direction. The rate-direction relationship approximates a triangular function, with the peak firing rate at the peak of the triangle. Firing outside the preferred range (eg. 90-130 degrees) approaches 0 Hz.

2) This basic set of properties is stable for a cell, and is not affected by darkness, environmental changes, or the head facing in the appropriate direction for a long time.
3) In different environments, the preferred direction may change, but as stated the basic tuning properties remain. Of some importance to the study of this thesis, early reports at least indicated that a given HD cell will tend to show different directional tuning in different shapes in identical laboratory conditions to that used for testing place cells. Thus in Taube et al (1990b), compared to a baseline reference direction in a circle, 8 out of 10 cells changed their preferred direction by at least 78 degrees in a rectangle, and 3 out of 8 changed their direction in a square. It may be speculated that the aspect ratio change in the rectangle is more obvious to the rat.

In a subsequent study (Golob and Taube, 1997), which it must be stressed examined HD cells in hippocampal lesioned animals, only 2 out of 11 HD cells changed their preferred direction by more than 18 degrees in going from a circle to a square. Again, more violent shape changes, from circle to triangle or pentagon, caused much more violent changes in directional tuning (all 17 cells tested, by at least 36 degrees). This study was composed of 11 Presubicular, and 10 anterior thalamic cells. No breakdown was given for cells involved in the shape-change component.

4) No exception has been found, to my knowledge, to the rule that an HD cell fires in all environments (so long as there is some opportunity for the animal to face in the appropriate direction.)

5) Generally, experiments involving rotation, such as on cue-card control, tend to show that HD cell tuning behaviour exactly mirrors the angular location behaviour of place cells (Taube, 1998).
D) INTERACTIONS BETWEEN HIPPOCAMPAL CELLS AND PRESUBICULAR CELLS

I take the view that the data up to now show that while the hippocampal and presubicular systems will be found in future studies to be to some extent parallel, and to some extent interactive, the presubiculum (and other regions on which it is dependent) controls the hippocampus more than the other way around, and is more independent of the hippocampus. In my view the data are entirely compatible with the strong statement that the presubiculum has primacy in cause-effect relationships. It must be said that this view is not universally shared. The following points can only in part support this view.

1) When the hippocampus is lesioned, HD cells are easily found, in proportions similar to those in unlesioned animals, with various parameters associated with directional tuning curves unchanged (Golob and Taube, 1997).

2) Moreover, the particular preferred direction of an HD cell in a new environment, in a hippocampus-lesioned animal, generally remains stable over a few days. This study is far from perfect (the lack of a control group being an obvious lacuna), but the stability, while it may not have been as good as in control animals, was in itself not that different from the stability of the HD cells in the familiar environment (experienced before lesions).

3) A Preliminary report indicates that when the presubiculum or anterior thalamus is lesioned, place fields become far more directional, even in an open field (Archey et al, 1997). This result was more pronounced in animals with anterior thalamic lesions. There was also the suggestion, of course an inconclusive finding by itself, that place
cells were harder to find. Furthermore, in conditions when a cue card was removed, place fields sometimes rotated to a new position (Dudchenko et al 1995; Archey et al, 1997): this phenomenon does not usually occur in intact animals (Muller and Kubie, 1987a; Bostock et al, 1991)
This chapter can pay only brief attention to the many theories and models associated with hippocampal function. As the original hippocampal cognitive map theory of O'Keefe and Nadel has been treated in Chapter 1, this is not further mentioned here, except in reference to other theories. The chapter begins with some models associated with spatial accounts of hippocampal function, and goes on to consider theories of hippocampal function which assume that the hippocampus computes more than spatial information. It must be emphasised that many theories are not even mentioned. These include those theories which have characterised the hippocampus’ defining role in memory in terms of providing a temporary memory store. The focus is on those theories which seem to be the most influential currently.

Spatial theories and models: place field formation and navigation

The following briefly discusses formalised models associated with spatial mapping functions and spatial problem solving. Only a few models are mentioned, the focus being on those which go beyond place field formation and consider some form of actual behaviour (elementary navigation).

Models of navigation

Distances-between-field peaks and synaptic weights - The Cognitive Graph model

The Cognitive Graph model (Muller et al, 1991: Muller et al, 1996, Muller and Stead, 1996) relies crucially on the idea that distances between fields are represented entirely by synaptic strength contact between neurons (only CA3 neurons are considered).
(This general theme is becoming increasingly common - eg. in Tsodyks’ (1999) model of spatial maps, not considered here, the strength of synaptic interaction between the neurons depends on the distance between their place fields.)

The graph model is grounded by three basic features, which are more or less facts: CA3 place cells, an LTP-like process, and dense CA3-CA3 connectivity. Then, it is assumed that those place cells with overlapping fields make strong contacts with each other, and those with non-overlapping fields make weak contacts with each other. Graph-theory, a form of mathematics associated with algorithms for extracting geodesics, is applied to the network of nodes (CA3 cells) and edges (axon branches). The extraction of the geodesic is applied after some learning.

The model’s successes include the simple demonstration that the network implicitly contains in its structure pseudo-cognitive map behaviour. In particular, with very few additional assumptions, it can deal with detours and short cuts (see Chapter 1 for the importance of these behaviours theoretically). There is indeed a clear prediction relating to short cuts - the animal must explore the altered area first. The model has no parallel of “insight” in these circumstances. The model cannot arrive at the solution of the short-cut near its starting point. There is no Tolmanian “expectation” of the environmental set up as a whole.

This raises an interesting issue relating to one of the central themes of this thesis: that we should try to work out what are the specifically hippocampal-dependent learning paradigms, and what are specifically hippocampal changes in learning. The cognitive graph model is a model of incidental learning in many respects, but it does not imply
fully cognitive-map behaviour as originally conceived. This is potentially very interesting. It can appear to deal with some of the problems related to latent learning that other S-R models cannot (Brown and Sharp, 1995; Sharp et al, 1996).

But the model as it stands has weaknesses, and if the model is to develop, it must surely deal with the two lacunae - an output, and a notion of goal-directed behaviour. It is a common criticism of the model that there is no obvious way to understand how the information in the CA3 network is read out by another system. Secondly, how does the destination come in to the model? Thus in Muller and Stead (1996), we read “a starting and a final node are selected.” Future work should perhaps try to incorporate an explicit mechanism for selecting the final node. One imagines that this is not complicated to model, but experimental support for the mechanism used might be lacking, and a clear testable prediction might emerge.

The model is interesting and well-motivated in as much as it tries to deal simply with known facts about the hippocampal system (place cells, LTP), to model known facts about behaviour (incidental learning, detour and short-cut behaviour).

S-R models: Brown and Sharp, 1995; Sharp et al, 1996

The same cannot be easily be said for many S-R models, such as those of Sharp and colleagues (Brown and Sharp, 1995; Sharp et al, 1996). It is far from clear why models of hippocampal navigation should be constructed which knowingly fail to account for those aspects of hippocampal function which are generally assumed to be dependent on it: latent learning-related behaviours. A few workers have raised doubts about the facts of incidental learning (short-cuts: Sutherland et al, 1987; latent
learning: Whishaw, 1991), and this remains a very underexplored experimental paradigm, given its theoretical importance. But the balance of evidence is not in favour of the doubters, and Brown and Sharp do not refer to any studies suggesting problems with the latent learning idea.

The best thing about the model of Sharp and colleagues is that it is simple, and is actually simulated. The architecture consists of sensory cells, place cells, head-direction cells, and motor cells, and the learning rules are conventional. There is no obvious shelving of features for later; the model can explain simple navigation in the water maze as it stands.

Its basic weakness, and that of others like it, has already been implied above. The model cannot learn anything without reward. A rat which has explored an environment without a goal for 1 hour has no advantage over one which has been in it for a second. It is a purely instrumental learning model.

The fact that learning is so reward-driven has implications for the way it executes certain behaviours it is explicitly modelling, as well as that it simply cannot execute other behaviours. The behaviour originally executed towards a goal is a powerful determinant of future behaviour. Burgess and O'Keefe (1996) point out that if stereotyped routes, such as clockwise spiral to the goal, were initially taken, future routes would be unduly affected by these initial paths. This is a classic Tolmanian point about the lack of flexibility in simple S-R systems.

The models of Burgess, O'Keefe and colleagues
Burgess and colleagues have devoted much effort to modelling details of place field firing and navigation (Burgess et al, 1994; 1996, 1997; 1998; 2000; Burgess and O'Keefe, 1996; Hartley et al, 2000; O'Keefe, 1990, 1991; O'Keefe and Burgess, 1996). These studies embrace several known features of hippocampal formation cell firing, including detailed simulations of place fields and their input, the phase shift phenomenon, and head-direction cells. The attempt to model latent learning, and behaviour thought to depend on it, such as quick navigation to a goal, and detour behaviour, is explicit. The model has also been used to investigate behaviour under conditions where the shape of the environment is changed. Finally, the model has been physically implemented using a robot (Burgess et al, 1997; 2000).

One of the central ways in which the model (Burgess et al, 2000 is the combined all-features version) differs from other models concerns activity round the goal. The model incorporates goal cells (assumed to reside in the subiculum), and assumes that whenever a rat encounters a goal location, it turns around to face in several different compass directions. Every goal location (in principle there can be more than one in an environment) is represented by a set of goal cells, each associated with a particular goal and with a particular head-direction. Goal cells have inputs from the head-direction and reward systems such that the connections from place cells to goal cells can be modified whenever the rat encounters the goal and faces in the appropriate direction. The modifications of connectivity occurs at a particular phase of the theta cycle, and switches on connections from place cells active at that time. The direction to a goal location can thus be represented by a population vector of a set of goal cells. (The model does not, unlike Brown and Sharp's, incorporate a read-out to a motor system interpreting the population vector.)
These goal-associated features are not purely arbitrary. Pavlides et al (1988) found that LTP induction is affected by the theta phase at stimulation. Behaviourally, as Burgess et al (2000) point out, “rats’ performance in the water maze is impaired when they are prevented from looking around at the goal location (Arolfo et al, 1994) and improved when they are allowed to (Keith and McVety, 1988).” The goal cells are of course a prediction of the model.

The only obvious weakness of the model is one shared by all models: what happens in different environments? The model does not address situations of remapping in different environments.

Modelling different environments

Different environments are not incorporated into the framework of any of the above models, except that Burgess et al, 2000 explicitly models behaviour in situations where only partial “remapping” occurs. This is not really a fault of the modellers: as this thesis may make clear at points, the hippocampal coding of different environments is not well understood. The issue of different environments is likely to affect some models more than others however. Ingenuity may be required to build multiple maps coherently into the cognitive graph model.

One of the features of the models of Burgess and colleagues since (O’Keefe and Burgess, 1996) is precisely related to the finding that there can be regularity in coding of environments which differ only in shape, at least initially. Hartley et al (2000), developed partly in response to the work in this thesis as well as that of O’Keefe and
Burgess (1996), can be considered to be a predictive model of what fields look like in environments differing in size and shape. The features of the model can then be incorporated into the overall model (Burgess et al, 2000). Perhaps the best feature of the place-field component of the model was its prediction of firing related to barriers.

Previously Muller and Kubie (1987) had shown how introducing barriers into or near fields had the effect of reducing firing rate. On the basis of the model in O'Keefe and Burgess, in turn inspired by empirical observations, Burgess and O'Keefe predicted that in some circumstances, the barrier might act as a wall, and thus that a place cell firing near a wall might develop two fields in these circumstances. (See Figure 8i in this thesis). It has not been possible to present all the data relating to predictions of the basic model, and its elaboration in Hartley et al (2000), but Chapter 8 briefly considers some of the predictions of the model. While the creation of extra fields with a barrier is not fully understood, the basic point is that a model was used to make a prediction of an observation not previously conceived of in the literature.

Non-spatial accounts of hippocampal function

In this section, three types of theory are mentioned: the configural learning theory of Sutherland and Rudy, the declarative theory of Squire, and the relational declarative theory of Eichenbaum and Cohen.

Configural learning theory (eg. Sutherland and Rudy, 1989; Rudy and Sutherland, 1994)

Configural learning theory is an explicitly associationist theory of conditioned learning. Here, hippocampal learning relies on reinforcement histories. These authors
argue that the hippocampus encodes configural representations of elemental cues, and not just the elemental cues. (In practice, it seems difficult to know how simple a cue has to be in order to be elemental. Many foods must have different, salient smells to rats in them. Is the food item configural or elemental?) Thus if an animal experiences two elemental cues such as an apple, and an orange, a configural association will be made in the hippocampus of the conjunction “apple-orange”. The point of the configural association is that it can disambiguate reinforcement histories. Apple may suggest reward, orange may suggest punishment, “apple-orange” may suggest any outcome, and storage of this conjunction prevents interference from the elemental reward/punishment associations.

A very important point is that the configural conjunction is then a single cue. Configural representations of many elements are still a single cue. In the relational theory of Eichenbaum and Cohen, discussed below, the hippocampal relational network “maintains independent representations for stimulus items and events and connects them by the relevant relational linkage in memory space” (Eichenbaum, 1994).

The theory has the merit of being well-specified and falsifiable, and indeed the theory has to many observers been falsified with a large number of studies failing to show hippocampal-lesioned animal deficits in such tasks as negative-patterning, and transverse-patterning (eg. Jarrard, 1993). In practice, the problem of defining an elemental cue has not really surfaced, because of readily obvious falsifications of the theory. This theory was included in this summary sketch because it makes such clear
predictions, and continues to generate hypothesis-testing experiments in the conditioning field.

**Declarative theory (eg. Cohen and Squire, 1980; Squire, 1987; Squire, 1994)**

This account of hippocampal function emphasised the distinction between declarative memory and procedural memory (Cohen and Squire, 1980). These distinctions map on respectively to distinctions later made between explicit and implicit memory (eg. Schacter, 1987; 1994). Later it became obvious that there were many types of learning system not well characterised as procedural and the term "non-declarative" learning has been used instead to characterise the non-hippocampal learning systems. Non-declarative learning includes the learning processes involved in acquiring skills and forming habits, priming, simple classical conditioning, and nonassociative learning. Like other theories of hippocampal function, notably cognitive map theory (O'Keefe and Nadel, 1978), and relational theory (Eichenbaum, 1994), the declarative theory emphasises rapidity, flexibility, and the richness of hippocampal memory. Conscious recollection is a further key component of declarative memory, and one that defines the theory. Thus for Squire, hippocampal learning and memory is expected to be "fast, accessible to conscious recollection, and flexible, ie. available to multiple response systems" (Squire, 1994). This account was based more on the human and clinical literature on amnesia, but virtually all hippocampal theorists predict continuity between animals and humans and these authors have not been exceptions. In the animal literature, the task most accepted as the declarative-tapping task is the non-match to sample task (with various delays). Other recognition memory tasks are also used in testing the declarative theory.
Declarative theory recognises the episodic and semantic distinction (Tulving, 1985), but predicts that the declarative hippocampal region system is involved in both types of memory. Accordingly, studies that suggest purely episodic deficits, with preserved semantic learning, for patients with bilateral hippocampal damage, are problematic for the declarative theory (Vargha-Khadem et al, 1997).

With regards to the animal literature, the vast majority of studies using restricted ibotenate lesions of hippocampus have failed to support a role for the hippocampus in non-match to sample tasks and recognition memory tasks (eg. see references in Jarrard, 1993; Mumby et al, 1996; O'Keefe, 1993). Furthermore, Mumby et al, (1996) helped to explain previous recognition deficits associated with ischaemic hippocampal lesions (Zola-Morgan et al, 1986; 1992) by showing that ischaemic-induced hippocampal rats showed recognition memory deficits but when their hippocampi were removed after ischaemia, their performance was unimpaired.

The issue remains somewhat controversial and Squire and collaborators have recently reinvestigated this issue in monkeys, and re-analysed old data, to suggest that damage limited to hippocampal region (CA fields, dentate gyrus, and subiculum) impair recognition memory at longer delays (Zola et al, 2000). Nadel (eg. 1994) has pointed out that during the longer delays some of the animals in this recent re-analysis were taken to another room, and then returned, and a failure to recognise the original testing context may be partly responsible for the deficit.

One of the other features of this theory is its prediction about consolidation. The theory has arguably been more successful here. Squire’s theory, based on the clinical
literature on retrograde amnesia, has emphasised the idea that hippocampal storage is
time-limited and that neocortical areas gradually acquire memory traces stored in
hippocampus, while any given hippocampal memory store decays. In essence, what
was in the past a hippocampal-dependent memory can become a neocortical-
dependent, hippocampal-independent memory. Using the consensus-based view that
the hippocampus is important for knowledge of allocentric spatial layouts, two one-
subject studies have recently shown intact remote spatial memory of this type in
patients with profound hippocampal damage (Teng and Squire, 1999; Rosenbaum et
al, 2000). The data in such one-patient studies must be regarded as preliminary. It is
possible that the spatial learning acquired by these patients is not cognitive-map like,
and that parietal and frontal cortex for instance can support some of the learning
tapped by the tasks used to assess spatial layout knowledge. Rosenbaum et al (2000)
have argued that their data does not support a consolidation view because their patient
did have a deficit in remembering visual location details. Such an argument would be
strengthened if the authors could show that this deficit was related to the patient’s
hippocampal damage.

Relational declarative theory (eg. Cohen and Eichenbaum, 1993; Eichenbaum, 1994;
Eichenbaum et al, 1999)
This account of hippocampal memory function has obvious similarities with the
original declarative account of Cohen and Squire, and Cohen has participated in the
development of the theory. Perhaps the main contribution of Eichenbaum’s theoretical
efforts has been to frame the theory in terms of the animal literature, and to introduce
olfactory-based tasks which test the theory.
Despite quite long theoretical exercises (Cohen and Eichenbaum (1993) is a book, Eichenbaum (1994) is over 50 pages) the specifically relational-emphasising aspects of the theory are arguably not very well specified, certainly not in comparison to the accounts of O'Keefe and Nadel, Rudy and Sutherland, and Squire. It is not always easy to work out how the theory can be falsified.

Eichenbaum and colleagues have emphasised that the hippocampal system supports a relational representation of items in memory, and that “a critical property of the hippocampal-dependent dependent memory system is representation flexibility, a quality that permits inferential use of memories in novel situations” (Eichenbaum, 1994: his italics). Eichenbaum has pointed to the antecedents of his learning account in the work of William James, Bartlett, and Tolman. Just as Tolman’s field-relations and field-expectancies were, Tolman felt, applicable to other modalities than space, so Eichenbaum considers the cognitive map theory of O’Keefe and Nadel only a special case of a more general relational learning function.

In my view, the 1994 paper (Eichenbaum, 1994) is the most concrete of the various theoretical statements of Eichenbaum, and the following is selected from that part of this paper specifically relating to predictions:

"In accord with our view of the hippocampal system as involved in memory representation for virtually any kind of learning, the first prediction from behavioral physiology is that hippocampal neurons will be activated by the key cues in any behavioral task examined, including during behaviors and in tasks in which no performance impairment is observed after hippocampal damage. [...]"
The second prediction from behavioral physiology is that the activity of single hippocampal neurons will reflect particular conjunctions and configurations of specific items relevant to the behavioral task at hand. [...] Finally, in accord with our view that hippocampal representations do not involve procedural aspects of behavioral performance, the third prediction from behavioral physiology is that hippocampal neurons will not fire in relation to simple sensory or motor events.” (Eichenbaum, 1994: his italics)

In my view, the first prediction is extremely hard to test. How is one to predict what the “key cues” are? Further, if one key cue is a shock for instance, it is reasonable to assume that some hippocampal neurons will reflect arousal from brainstem areas. If further this occurs during a task which is not hippocampus-dependent, as the prediction makes clear is possible, in what sense is the prediction falsifiable?

The second prediction is fairly clear, but does not differentiate the theory from others. Where one of the cues is related to location, this prediction suggests the properties of O'Keefe's misplace cells (see chapter 4). Any version of configural theory would also predict cells which fire for conjunctive cues. What is missing from the prediction is how to know what conjunctions will not be encoded. If all are encoded, then why aren't some conjunctions ever seen? For instance, why aren't some place cells seen with fields along the entire edge of a circle or square? If the conjunctions are only of cues relating somehow to reinforcement, this is not reconcilable with the incidental learning aspects of the relational theory.
The third prediction seems to be at odds with the first prediction. Won’t some simple sensory events form “key cues”? At any rate, the third prediction appears to be falsified by the paper (Wood et al, 1999) on which Eichenbaum is the last author, and that has been viewed as providing the most important unit-recording support yet for Eichenbaum’s relational theory. 91 cells out of 127 recorded had statistically significant correlates. 10 were “odour” cells, and there were 2 “odour and match/non-match” cells, 4 “Position and odour” cells, and 4 “Position and Odour and match/non-match” cells. In other words, there were as many odour cells, cells responsive to presumably “simple sensory events” as there were odour-related cells which reflected “particular conjunctions and configurations of specific items relevant to the behavioral task at hand”. This is not expected by the theory.

It is explicitly stated that “odour cells discriminated best and worst odours at all positions”. Their Figure 2 shows a cell which basically only fires to odour 5, of 9 used, with slightly increased firing for odour 8. The simplest interpretation of such reported correlates is that these “odour” cells reflect a “simple sensory event”. (Perhaps it will be argued that the correlate reflects a discriminatory property. But if this is argued, what is a simple sensory response, and again, what are the non-relational boundaries? When is something not-relational?) But if the conjunctions are so important, why are there not more (and many more at that) odour/conjunction cells than odour cells, especially since there are three types of conjunction involving odour?
Relational theory tells us that, with regards to the second prediction above about conjunctional coding, "the specific nature of the conjunctions or configurations among essential cues reflected by neural activity will vary across tasks depending on the particular types of relationships relevant to accurate performance" (Eichenbaum, 1994: my italics). What is the task? The task is "an odour-guided, continuous, non-matching-to-sample task", and the mean task-response during recording was 96.8% correct (Wood et al, 1999). It seems to follow inescapably from Eichenbaum’s theoretical formulation that: since position was not relevant to accurate performance, and since performance was indeed highly accurate, then odour and match/non-match relationships should be the most important conjunctions and receive prominent hippocampal encoding, while position and position-related conjunctions, and position and odour and match/non-match conjunctions, should be less prominent in hippocampal encoding. The number of cells encoding odour/match/non-match conjunctions was 2! This is an order of magnitude lower than the number of cells with position conjunctions. Why is there so much more encoding of these distractor-type, success-irrelevant relationships, especially given the very dense olfactory input to the rodent hippocampus? The data are not even superficially accounted for by the theory, but go against the predictions.

To the extent that the relational account goes beyond data-labelling and is a theory, with predictions, its author’s own data falsifies it in a simple way; to the extent that it simply says hippocampus can encode non-spatial variables, it has nothing obvious to offer that other accounts do not. Nevertheless, this account of hippocampal function seems rapidly to be gaining support.
CHAPTER 6
MATERIALS AND METHODS

4 sets of experiments are described in the present thesis. These were:

a) the pilot study; b) Experiment 1A; c) Experiment 1B; and d) Experiment 2.

This chapter focuses on general procedures. Chapters 7, 8, and 9 include description of methods and procedures specific to the pilot study, experiment 1, and experiment 2, respectively.

General Procedures for all experiments

Subjects

In total, 15 male Lister hooded rats were used in the experiments reported in the present thesis. Excluding the pilot study, 10 rats were used, in experiments 1A, 1B, and 2. Rats weighed 280-425g at time of surgery, were housed singly in Perspex cages, and maintained on a 12:12 hour light:dark schedule, with lights off at 3pm.

Rats were weighed daily, and maintained at about 85% or more of their free feeding weight. As described below, rats were implanted with electrodes before the various experiments, and no selection procedure was used to weed out slow movers, excessive groomers and so on. This is in contrast to the weeding out procedures reported in Bostock et al (1991) and in the Muller laboratory in general.

The subjects in the various experiments were as follows.

Pilot: r962, r975, r974, r961, r956, r966, and r977

Experiment 1a: r1004, r1020, r1039

Experiment 1b: r966, r977 (both animals used in pilot), r995, r1062
Experiment 2: **r1029, r1077, r1079**

In all, 10 rats were used in experiments 1 and 2 (in **bold** above).

**Electrodes and Microdrives - basic details**

The electrodes used were tetrodes (O’Keefe and Recce, 1993), and at least two tetrodes were implanted in each animal. Each tetrode consisted of four twisted strands of HM-L-coated platinum-iridium wire (90%/10%) (California Fine Wire), either 17 or 25 μm in diameter. (No consistent differences in results were observed between the 17 and 25 μm tetrodes.)

13 out of 15 animals were implanted with the standard “poor lady” microdrive. 2 animals were implanted with one large microdrive. The precise breakdown is as follows. 10 animals were implanted using one microdrive with two tetrodes. Regarding the other 5: one had four tetrodes using one microdrive (r1079); two had four tetrodes using two microdrives with two tetrodes each in each hemisphere (r1062, r1077); two had eight tetrodes using one large microdrive (r1004, r1020).

Further details about the microdrives and tetrodes are given below.

**Microdrives - further details**

Figure 6a is a diagram of the standard “poor lady” microdrive, showing its mechanical features. Figure 6a shows the drive position as if the electrodes had been introduced into the brain, and the tips of the electrodes were 1.5 mm below the surface of the skull. During surgery the feet of the drive, and the sleeve around the cannula encircling the electrodes, are affixed to the skull using dental cement.
The "poor lady" microdrive

Figure 6a. Diagram showing basic features of the "poor lady" microdrive.

Most of the rats in the present study were implanted with this type of microdrive.

Dental cement is used to connect the cannula to the main screw and frame. After introducing the electrodes into the brain (eg 1.5mm as shown beneath the skull surface), dental cement is used to affix the sleeve and feet of the drive to the skull. The diagram does not show electrical wire connections from electrodes to the plug.

The original version of this drawing was done by John Huxter.
Mechanical features of the drive are briefly described. If we ignore the cannula on the right of the diagram in Figure 6a, there are basically two posts. These two posts are joined at the bottom (soldering joint near word “frame” in Figure 6a) and at the top (with a flange and nut). These two posts comprise the stationary part of the drive. One of the posts (right hand side in Figure 6a) bears a piece of tubing carrying a screw-thread (“main screw”). The dimensions of these two tubes are chosen such that the second tube can turn freely on the first without wobbling. Two small sections of heatshrink tubing are fitted onto the microdrive, one onto each post. When the heatshrink tubing is shrunk well, the tubing round the screw thread on the right-hand post becomes moulded to the thread in the process and forms a “nut”. Dental cement is then applied around these pieces of tubing. This forms the basis of the moveable part of the microdrive, and is cemented to the cannula (shown).

Not shown are the plug and connection wires mounted onto this moveable part of the microdrive, using dental cement. The standard set up is to mount 8 wires, 4 on each side, onto the moveable part. However, 16 wires, 8 on each side were mounted onto it for r1079. The wires are soldered at the plug end, so that electrical signals in the wires can reach the headstage, when the headstage is connected to the plugs. At the other end, the wires are cut off to form post: each wire of the tetrode is tightly wound round one of the four posts relating to that tetrode.

When the feet of the drive are fixed in space, turning the screw (“screw-turner”) will cause the moveable part of the drive to go up or down. A 360 degree turn of the screw moves the cannula and electrodes down by 200 microns. In general, no turn is made
of less than 45 degrees (ie. 25 microns). The point of the spring (see top of right-hand post “spring”) is to confer smoothness and stability on the movements of the screw.

Broadly similar principles are behind the design of the large microdrive used for r1004 and r1020, onto which 8 tetrodes can be loaded. However, because it is difficult to get many tetrodes all in the pyramidal layer simultaneously, the large microdrive was designed (by John O’Keefe and David Edwards) with 8 separate screws; this allows for independent movement of each tetrode. The large microdrive contains 8 posts arranged in a circle. The tetrodes connected to each screw are all fed, and move, through a circular array of individual cannulae. At the bottom of the drive, the tetrodes fit into a sleeve, which is like an 8-bullet gun revolver. As with the standard small “poor lady” microdrive, in surgery dental cement fixes the feet of the drive and the sleeve to the skull.

Tetrodes - further details

Tetrodes were constructed by twisting four individual fine pieces of wire together. This is done by taking one piece of wire, sticking the ends together, and creating two loops. The top of the two loops were hung on a post, and the wires were twisted clockwise (about 15-20 turns per mm). The top of the loops are cut to form free ends. These free ends are tied around the posts of the drive. The other end is cut to form the tetrode tips which will be implanted into the brain.

For experiments 1 and 2, tetrodes used in the standard microdrive, which does not allow for independent movement of each tetrode, were sometimes made and cut so that the tips of each tetrode were located at similar dorsoventral levels (eg. r1079,
In other words, the aim was to achieve a state of recording, at some stage in the experiment, with two or more tetrodes recording from the same pyramidal layer simultaneously. This was simply to increase the potential yield from any one microdrive.

**Surgery**

Rats always underwent surgical implantation of movable microelectrodes *before* the given experiment. Rats were anaesthetized using isoflurane and nitrous oxide, and given an i.m. injection of buprenorphine (45µg) for intra- and postoperative analgesia, and an s.c. injection of enrofloxacin (2.5mg) as a prophylactic antibiotic.

Rats were first anaesthetized using the gas preparation as follows: Oxygen at 3 litres per minute, with isoflurane at 3% of the gas volume. Before the earbars are placed in the animal, nitrous oxide is introduced at 3 litres per minute, and the oxygen flow is reduced to 1.5 litres per minute. Around this point, the i.m. injection of buprenorphine and s.c. injection of enrofloxacin are given. The percentage of isoflurane is gradually decreased throughout surgery, which usually ends at levels of 0.5 to 1.2%.

The target coordinates for electrodes in animals implanted with a single microdrive were centred, in either hemisphere, as follows: AP -3.8 to -4.0 mm; ML 2.4 to 2.7 mm, (both AP and ML relative to bregma); DV 1.5 to 1.7 below pial surface. For animals implanted with two microdrives, the two targets were a) more anterior and medial (AP -3.0 to 3.3 mm; ML 1.3 to 2.0mm) and b) more posterior and lateral (AP -4.0 to -4.8 mm; ML 2.5 to 3.0 mm) with the same DV distance. A 2mm diameter hole was drilled into the skull above the target coordinates.
The tetrode-microdrive assembly was fixed to the skull by a) screwing in jeweller’s screws into the skull, and b) applying dental acrylic around the feet of the assembly and on the skull and screws. One screw soldered to a gold Amphenol pin served as the ground attachment. A plastic screw, screw end down, is cemented to the skull, providing the attachment for the LED-holder part of the headstage. Other plastic screws are also cemented where necessary to protect the drive from any grooming movements by the animal.

**Unit recording**

Each rat was connected to the recording equipment via a headstage (or two headstages) which fitted onto the plug(s) of the microdrive(s). The headstages used were unity-gain buffer amplifiers; the implanted electrodes were AC-coupled to these amplifiers, which served to isolate the electrodes from the wires carrying their signals to the recording system. One or two small, infrared light-emitting diodes (LEDs) were attached to the rat (anchored via a plastic screw cemented to the rats' skull during surgery) for the purpose of tracking the rats' movement with a video camera and position-detection hardware. Lightweight hearing-aid wires 2 to 3 metres in length connected the headstage(s) to a preamplifier (gain 1000). The outputs of the latter passed through a switching matrix, and then to the filters and amplifiers of the recording system. The switching matrix is an array of analogue switches to which all of the electrode signal inputs and all the amplifier boards are connected. Under software control, the switching matrix allows the user to control which signals get recorded differentially with respect to which other signals.
Signals were amplified (c. 15-50 thousand times) and bandpass filtered (500 Hz-7 kHz). Each of the four channels of a given tetrode was recorded differentially with respect to a channel on another tetrode. Differential recording is now a standard technique, used to subtract noise and artifact from the signal. If two channels pick up the same artifact signal, and a cellular signal, differential recording will pick up only the cellular signal. Occasionally (rarely), it was necessary to record from two "active" tetrodes where each was referenced to a channel on the other. Fortuitously, in all such cases, no obviously overlapping place fields were recorded, and in any event, the data in this thesis will not be used for correlation purposes. Each channel was continuously monitored at 20-μs intervals, and potentials were captured with 50 sampling points per channel (1ms, with 200 μs pre-threshold and 800 μs post-threshold) whenever the signal from any of the pre-specified recording channels exceeded a given threshold set by the experimenter (ie when a presumptive neuronal spike occurred). Each spike event was spatiotemporally stamped with: a) the time since the start of the recording, and b) the x, y location of the LED, or LEDs, as determined by the position-detecting hardware (position sampling rate 50Hz). All such data were stored on a hard drive and transferred to a SUN workstation, for later analysis. Most of the animals were recorded with two LEDs, and thus head-direction information, as well as x,y location could be determined.

All the microdrives, headstages, preamplifiers, tracking and recording systems, were purpose built (John O'Keefe Laboratory members; Gignomai Ltd; Axona Ltd).
Long-term recording

One aim in experiments 1b and 2 was to be able to follow cells over several days, and even weeks if possible. This was of course to try to observe experience-dependent or learning-based changes at the level of the individual cell. Accordingly, once amplifier gains and thresholds were set for a tetrode, they were changed as little as possible. This helped to identify a cell recorded over a long period. In order to achieve a high level of stability, recording did not usually begin until several weeks after the surgical implant. In order to prevent accidental movement of the electrodes, blue tack was sometimes affixed to the screw-turner on the microdrive(s). Efforts to achieve long-term stability were particularly important in experiment 2.

Screening for activity and ensuring stability

Rats were screened for cell activity on the holding platform, and the experimenter checked for stable place cells on the holding platform, before recording. Screening generally did not begin until at least a week after surgery. As described in Chapter 3, on the physiology of the hippocampus, various EEG and cell firing characteristics can be used to determine the whereabouts of the electrodes in the hippocampus. Particular attention was paid to the high-frequency “ripples” state, seen when the animal is still and at low arousal levels (including sleep). The electrodes were moved down towards CA1 in multiples of 50 or 100 micron steps until signs of low amplitude “ripples” were seen. When ripples were seen, the electrodes were moved down in 25 micron steps. The basic idea was to leave the electrodes above the layer or simply left at that dorsoventral level to gradually drift into the CA1 layer. At some point, place cell activity would be seen on the holding platform. This was almost never recorded, but
the experimenter would make rough diagrams of place fields based on this activity.

Once stable fields were seen over days, recording would begin.
General Laboratory layout - Experiments 1 and 2

Differently-shaped walled boxes placed in same position in curtained environment

Figure 6b Laboratory layout for experiments 1 and 2.

HP = holding platform. X marks the centre of the curtained environment. Either a Square-walled box, or a circular-walled box, is placed in the curtained environment, centred on X. The camera viewing area is kept constant.

Rat is always brought into the curtained environment through the lab-South Point of entry, head facing lab-North, and placed at X before being released.
Laboratory layouts and testing procedures - general

Precise details, with further Figures, of the environments and procedures used in each experiment are given in chapters 7, 8, and 9. Here, the general laboratory layout is shown for experiments 1 and 2 in Figure 6b. (The pilot study was conducted in a different laboratory). In each experiment, recording took place in differently-shaped walled boxes: one square-walled, one circular walled.

Trial timing, sequence and alternation - general comments

Trials always alternated between circle and square shapes, except in the training phase of experiment 1B described in chapter 8. Trials were always recorded for 8 minutes in the given square, and 10 minutes in the given circle (these times being proportional to the different surface areas of the two shapes). In some probe trials, an extra minute or so was added to the trial length to allow the rat to “adjust” to the new environment. In the main experiments, the intertrial interval (ITI) was rigorously maintained between 20-25 minutes. Very few ITIs were longer or shorter than this. The ITI on transfer/probe/further manipulation days was sometimes longer than 25 minutes. Generally, recording began within 0-15 seconds of placing the rat in the environment, and ended about 5-20 seconds before the rat was taken out of the environment. In other words, recording was performed for almost the entire duration of the animal’s experience in the environments. Each rat was tested at approximately the same time of day, though this time could differ for each of the rats within the same experimental series.
Procedures for bringing each rat in and out of the environments (Exps. 1 & 2)

Rats were brought in and out of the laboratory and the environments according to standard procedures. As mentioned, ITIs were strictly controlled. At the beginning of each day, the experimental rat was brought into the laboratory as follows. It was always brought around the outside of the of the black curtains (on the room East side - See Figure 6b), which were always drawn so that the rat could not see inside; then it was placed on the holding platform (HP in Figure 6b). The time spent on the holding platform before trials began did vary, but was always at least 20 minutes. The rat was placed into the given walled box after being carried through an opening in the south side of the black curtains (Point of Entry in Figure 6b), and over the south side of the box. The rat was always placed into the box with its head facing laboratory north, and it was always placed at the centre of the box, give or take a few centimetres. The only exceptions to this occurred in probe trials specifically examining direction sense. The animal was always on the holding platform between trials.

These procedures are in addition to the use of cue cards to polarise the environments (described fully in chapters 8 and 9 for experiments 1 and 2 respectively.) No attempt was made to mask sounds or other cues which could, in principle, help stabilise the head-direction system of the rat.

It should be noted that these procedures, designed to create stability in the head-direction system, are very different from those generally employed in the Muller laboratory (eg. Bostock et al, 1991; Fenton and Muller, 2000) where animals are taken out of the room between trials, and rats are introduced into the testing environment pseudo-randomly from four entry points in the curtains spaced 90 degrees apart.
There is no mention of these procedures in Quirk et al, 1992, but we assume they were similar to others in the Muller laboratory. Clearly, the procedures in the Muller laboratory are designed to create ideal directional control over the place cell firing by the intramaze cues. It is possible that the rats have less chance to appreciate the essential similarity of the position of the testing environments in the Muller-type set up.

**Environments – the “morph box”**

Recording took place in differently-shaped walled boxes. In all cases, the standard testing situation compared firing in square-walled vs circular-walled boxes. In the pilot study, and in experiments 1A and 1B, this was achieved using a “morph box”, presently described, from which several shapes could be constructed. In experiment 2, the morph box was used as a transfer-testing environment.

In order to create environments whose shape could be changed, without affecting other modalities such as olfaction, texture, colour (or shade), “morph boxes” were constructed (See Figure 6c). Each morph box consisted of 32 pieces of interlocking plastic tubing. To hide the joints, a layer of plastic wrapping tape was stuck to the inner surface of the walls, and then another layer of masking tape was stuck onto the plastic wrapping tape. (By itself, the plastic wrapping tape causes static problems). Two such morph boxes were constructed. For ease of reference, the morph box configured with circular walls is hereafter referred to as the *morph circle*, and the morph box configured with square walls is hereafter referred to as the *morph square*. 
Construction of the Morph Box

a) 32 individual pieces of plastic tubing

b) Pieces interlocked and inner surface covered by packing tape and masking tape to form deformable wall material

c) Variously-shaped walled enclosures formed from same material perimeter

d) When necessary, larger walled enclosures (e.g., large square d configuration) can be formed from 2 sets of such material

Figure 6c. Construction of the morph box.
Task

The task was similar to the pellet-chasing paradigm developed by Muller and Kubie (1987) and used by many others since. The only difference is that the rats foraged for grains of sweetened cooked rice. The rice was thrown by the experimenter into the environments, at a rate of about 1 to 3 per minute. Rice throwing was not done entirely pseudo-randomly but was sensitive, especially later on in the trial, to the locations the rat had already been to, in the interests of homogeneous coverage of the environment. The paths taken by the rat in the current trial were monitored (as described above - “Unit recording” section) and shown on screen. Either a colleague would report undersampled positions, or the experimenter would briefly create a gap in the curtains and look through the gap at the screen.

Data analysis

Data analysis – the isolation of single units from the multi-unit tetrode data

Creating individual clusters from the multi-unit data is known as cluster cutting or spike separation. The basic idea of cluster cutting from extracellular tetrode records has been validated in recent studies which explicitly compared intracellular and extracellular recording of hippocampal pyramidal cells in CA1 (Henze et al, 2000; Harris et al, 2000). Harris et al (2000) asked a group of naive and experienced experimenters to cut clusters of cells from the extracellular record. They then compared this to the intracellular record. Their general conclusions for our purposes here were that spike separation based on tetrode recording was markedly superior to that obtained from single electrode recording, and that so long as the spikes recorded were about 90-100 microvolts and above in peak-to-peak amplitude, the cutting was
very accurate, ie. individual clusters did belong to individual cells, and relatively few false negative and false positive errors were made. (Very few of the cells recorded in the present study had peak-to-peak amplitudes below 90-100 microvolts.) They did note that individual humans had consistent biases, in terms of their patterns of false positive errors (inclusion of spikes from cell(s) other than the cell in question) and false negative errors (omission of spikes from the cell in question). Each operator tended to have a particular bias towards one type of error. Accordingly, it is appropriate to give some brief comments about the cutting procedures used in this study.

Cluster cutting was performed manually on SUN Ultra workstations and computers emulating UNIX systems, using custom made software (TINT, written by Neil Burgess). No automatic cluster cutting algorithms were used. Collected waveforms were plotted and separated into clusters on the basis of peak-to-peak amplitudes, and waveform shape (strictly speaking, voltage heights at particular times).

The basic idea of tetrode cluster cutting, focusing on peak-to-peak amplitude at first for simplicity of description, is as follows. In tetrode recording, a cell produces four action potentials, one on each electrode channel. The peak-to-peak amplitudes of spikes are displayed on the axes of n-dimensional plots where they form separable clusters, since each cell tends to give a different profile of amplitudes over all the four channels compare to another cell. Each scatter plot contains all the recorded spikes. There are two axes. One axis represents the amplitude of the spikes on one channel, the other represents the amplitude of the spikes on another channel. Since there are four channels per tetrode, there are 6 scatter plots in all. The basic idea of cutting, in
essence, is that the experimenter draws a polygon around a well-isolated cluster. The TINT programme creates an ellipse from the polygon: this defines a cell. Different scatter plots are used to cut the clusters. A cluster well isolated on one scatter plot may not be so well-isolated on another scatter plot. Cells that are not well-isolated on the basis of peak-to-peak amplitudes (A) may be separated on the basis of waveform shape. In the TINT program, this is done by plotting the voltage of spikes at a particular timepoint (Vt). Accordingly, more dimensions can be added to the tetrode scatter plot space by plotting A vs Vt, Vt vs Vt, as well as A vs A described above. In practice, the present experimenter never used Vt vs Vt plots to separate cells. In order to reduce error, clusters that do not appear to be well-isolated are ignored.

It should be noted that cells can drift into, and out of, good isolatability, over days and weeks. Cells rejected one day may be acceptable on others. Thus it should not be automatically assumed that if the reported total number of cells analysed is very similar from one day to the next, that a very similar ensemble of cells is being sampled, (though this is often the case.) Generally, inspected rejected cells showed, as far as could be inferred, similar patterns to the population of selected cells.

Cluster cutting was not done on a trial-by-trial basis

In order to try to increase the objectivity of the cluster-cutting, with very few exceptions (usually due to one problematic channel) spikes were not cut on a trial-by-trial basis, but rather from a general template of ellipses derived for a given day. (See Figure 2cx, parts A), B), and C). These ellipses are certainly derived from the actual spikes cut in the various clusters. However, the precise set of the individual spikes is not used to define the presumptive cell. Rather, all the spike data loaded from one,
A) Loading Superimposed spike records.
For each tetrode, the raw spike records from one or more individual trials are loaded into a single file.

B) Manually Cutting the Superimposed spike record, & creating a template.
Cells are defined by manually drawing polygons round spike clusters. Programme derives ellipses modelling the cells. These ellipses form the daily template.

C) Automatic cutting of cells in individual trials.
Programme loads ellipses from the daily template, and cuts cells for each trial, one trial at a time. (3rd trial is shown.)
D) Example of the use of partially overlapping cells.
Both cells a and b fire in the circle. Cell b does not fire in the square. An artificial, “overlap” cell is cut according to the procedure shown on the opposite page. Sections I and II show the firing rate maps for the cells in the square (I) and circle (II). The right hand column in I and II shows raw spike-location data superimposed upon the firing rate maps. Section III shows, for each trial, 2 of the 6 available scatterplots plotting spikes in terms of peak-to-peak amplitude on two of four channels. The larger plot shows the spikes on channel 2 vs 4, the smaller plots show them on channel 1 vs 3.

Note that the firing of “cell b” in the square is in cell a’s field.

Small-interval dashed arrow indicates region of North-West region firing in “Overlap” cell map possibly due to cell b. Larger-interval dashed arrow indicates region of Central region of firing in cell b map possibly due to cell a.
two, three, or even four trials are superimposed, thus creating a generalised tetrode cluster space (see Part A of Figure 2cx). It is the template (in the form of a set of ellipses, each ellipse corresponding to one virtual cell) derived from this cluster space, that served to cut all the cells in all the trials on that day (See Parts B and C of Figure 2cx). It was not permitted to fine-tune a trial's template generated cluster cut, by cutting individual spikes, noise etc. If the cut seemed obviously misleading, it was only permitted to change the general template, and start again.

It was sometimes necessary to use cells which partially overlap in the superimposed tetrode scatterplot template space for a given day. (Note that this kind of cell will often be completely isolated from other cells on the tetrode it is recorded from, in the trial in which the cell fires.) This is necessary because otherwise one introduces a sampling bias against cells which only fire in one shape. Accordingly, the overlap of two cells was sometimes cut out and ignored. Of course, it implies a reduction in the reported absolute peak firing rate of the two cells in question. Part D of Figure 2cx shows an example of the use of partially overlapping cells. I would estimate that between 5% to 20% of cells may be affected in this way.

As stated above, shown by Harris et al (2000) human operators using manual cutting tend to have consistent biases. In my opinion, the cutting procedure used in the present study tends to produce more false negative than false positive errors.

Data analysis – derivation of place field characteristics

There is no standard protocol used to construct and present place fields. The procedures used in this thesis and described below follow, very closely, the protocols
used recently in the O'Keefe laboratory (O'Keefe and Burgess, 1996; Jeffery and O'Keefe, 1999). The software used is the previously mentioned TINT program (written by Neil Burgess).

To determine place field characteristics (e.g., peak firing rate, and location of peak firing rate), a boxcar averaging process was employed, which converts the raw spike data into false-colour contour firing rate maps. This process is described below, after considering the spatial framework used for analysis.

The camera viewing area was divided into a 64x64 bin grid system. The camera was an analogue camera, with digitisation performed by the tracker at 10 bit resolution. Thus its spatial view is divided into a theoretical maximum of 1024 x 1024 spatial units. In practice, the total possible camera viewing area is 768 pixels in the x dimension, and 574 pixels in the y dimension. A mask created by the software excluded all but 400 x 400 pixels. The environments are always centred, every trial at the centre of this 400 x 400 pixel coordinate frame. The analysis software (TINT) places all coordinate frames less than or equal to 512 x 512 pixels within a 512 x 512 viewing area. It is this “camera viewing area” that is divided into 64 x 64 bins. Accordingly 1 bin is 8 pixels long. Thus the minimal spatial unit in the TINT analysis software is 8 pixels, while the spatial resolution of the camera/tracker is 1 pixel. In experiment 1, 100 cm equals 300 pixels. In experiment 2 using a Trespa platform raised from the floor, 100 cm equals 332 pixels. (In practice, particularly with the “morph box” described below, the two-dimensional filled squares and circles “drawn” by the LEDs on the rats’ heads differed (within the same shape) by small amounts, but
The effects of smoothing

<table>
<thead>
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<th>1 No</th>
<th>3 No</th>
<th>5 No</th>
<th>5 Yes</th>
</tr>
</thead>
</table>

Figure 6d. The effects of smoothing on the derivation of place field characteristics.

Two place cells are shown here, one in brown (top two rows), and one in pink (bottom two rows). Each row represents the same trial, with different smoothing parameters.

In the left-hand column, bins where the cell fired are represented as square dots, superimposed upon the paths taken by the rat. The next four columns show the firing rate maps resulting from increasing levels of smoothing. The firing rate map in each bin is mapped as a false colour plot. The five colours are autoscaled so that each colour represents 20% of the peak rate (colours in descending order: red, yellow, green, light blue, dark blue). In the right-hand column linear interpolation between bins is applied. Note that interpolation has no effect on any place field characteristics, and is a purely visual aid.

Smoothing tends to reduce the peak firing rate, and can alter the aspect ratio of the field, as can be seen in the bottom two rows.
to give an idea of the binning system, the lengths of the sides in the square wooden
box (experiment 2) is 25-26 bins.)

The basic procedure for deriving firing rate maps is that for a given bin, the number of
spikes of each cell in that bin is divided by the rat’s dwell time in that bin, to give a
firing rate for each cell in that bin. The boxcar averaging or “smoothing” process is
then applied to these bins for each cell. Note that the place field is not derived by
dividing the smoothed spikes map by the smoothed dwell time map (which gives
subtly different results). Rather, as stated, smoothing is applied once the bins have
already been assigned initial firing rate values. Following previous work (O’Keefe
and Burgess, 1996; Jeffery and O’Keefe, 1999), the smoothing level used in this
thesis is 5 in the TINT program. The smoothing procedure applied is the standard
kind of averaging. Smoothing level 5 means that for each bin, a larger square with 5-
bin-long sides, whose centre is the current bin, is used to smooth the rate of that bin.
The current bin’s rate is the simple average of the rates of the 25 bins in the 25 bin-
large-square. This smoothing is applied to every bin in the environment, ie. the larger,
25-bin-in-area, smoothing squares are overlapping. The firing rate in each bin is
mapped as a colour or grey-scale plot, with linear interpolation between bins. The 5
colours or shades are autoscaled so that each represents 20% of the peak rate. In
descending order, bins with the highest rates are shown in red, then yellow, then
green, then light blue, then dark blue for the lowest rates. A white bin represents an
area that is unvisited.

The effects of smoothing
Field shapes and sizes vary, and while the parameters used for bins and smoothing have proved useful in previous work, the use of a universal procedure for all place fields has some "biases". An example of the effect of smoothing levels is shown in Figure 6d. As we would expect, smoothing decreases the peak firing rate. But note also that the large square smoothing method can partly obscure the shape of linear edge fields, such as the "crescent"-type fields frequently found in circular environments as previously reported by Muller and others (Muller and Kubie, 1987; Muller, 1996). Specifically, it tends to reduce the aspect ratio of linear edge fields. This does not really affect the results of this thesis, and it is hard to imagine a fairer alternative procedure for this dataset. In principle, one solution is to record for much longer, and apply less smoothing, but this was not deemed practical. At any rate, in this thesis, only the firing rate and the x,y position of the peak pixel (ie. at the centre of the peak bin), after smoothing, contributes to the analysis.

Data analysis - "silent" cells

Following the convention in (O'Keefe and Burgess, 1996; Jeffery and O'Keefe, 1999), only place fields with peak rates at or above 1.0 Hz are shown in firing rate maps, and a cell firing below 1.0 Hz is considered to be "silent" (Muller, 1996) in that environment. Clearly, any single cutoff point is arbitrary. The figure of 1.0 Hz was chosen as a threshold by O'Keefe in his recent experiments and collaborations based on earlier results in the 1970s where he found that the average firing rate of place cells over the whole environment over the whole recorded period was about 1.0 Hz (O'Keefe, personal communication). Of course, peak firing rate depends on smoothing, as Figure 6d shows, and bin size. In the experience of the present experimenter, at smoothing level 5, 1.0 Hz is more reasonable than some other cutoff
points. It is often observed that it is below around 1.0 Hz (using the above protocol) that a field apparently “breaks down” into several components, as if the cell is barely signalling above noise. Certainly, this is not always the case. Some cell-trials were rejected from the analysis of field-peaks despite seemingly clear localised firing with low-rate peaks.

Some standard definition of non-firing is required to correspond to the “virtual silence” of cells in one of two or more environments when remapping occurs (Muller, 1996). The use of a cutoff procedure obviates the need for naïve human observers to judge complex remapping (Bostock et al, 1991).

Data analysis - Preliminaries before firing patterns can be compared within and across shapes

Only cells which fired on more than one trial were included in the formal analysis. It was considered that little information could be gathered from say a cell which only fired on the first or last trial of the day, and thus in one shape only. Cells which fired on only one trial were, in any event, not common, but no quantification of the amount of cells excluded is provided.

Because the environments were always centered on the same point in the laboratory space and camera viewing area (see “x” in Figure 6b), the same coordinate grid could be used to compare trials. The experimenter was at pains to place the shapes in the same place from trial to trial, and inspection of the vertex files suggests this was successful. (A vertex file is a record of the x, y position of each of the four corners of a square made by the accumulated paths taken by a rat. The total path “fills in” a
square shape against a background of unvisited areas. Thus each square trial creates four x, y numbers corresponding to its four corners, North West, North East, South West, South East. Given that a circle is transformed into a square for analysis (see next section), this circle-transformed-into-a-square will also have four corners. The TINT software allows one to manually draw in vertices based on the paths made by the rat, if the automatic vertex file is not sufficiently precisely delineated.) Moreover, variability in placement was somewhat greater from day to day, than within a day, suiting the fact that the analysis is applied daily. Not all vertex files have been inspected, but a rough estimate from experiment 2 is that the mean daily trial-to-trial variability of a fixed point in the intrashape space is no more than 4-5 pixels, and the mean trial-to-trial day-to-day variability is no more than 7-8 pixels.

The above suggests it is very reasonable to use the coordinate grid of the camera viewing area to compare trial-to-trial field peaks within shapes, and across shapes, once a spatial transformation has been applied to convert the circle data into square data.

**Data analysis – comparison of trials in square and circle shapes - Spatial transformations**

In order to compare the firing fields in two different shapes, it is necessary to transform the data. This is a standard procedure, which was done in the studies of Quirk et al, (1992), Sharp (1997), and others. A spatial transformation algorithm was written (by Neil Burgess) which transforms the position data obtained from the rats. 

*This topological transformation was applied in one direction only, transforming the circle data into square data.* The transformation used is that which maps all points in
a cylinder into a square with the same centre and circumference. More precisely, each
tracked position in a cylinder is moved to the position in the square that is the same
fraction of the way from the centre to the edge of the environment. The
transformation is, formally, exactly like that used by the Muller and Sharp
laboratories except that it goes in the opposite direction. In Quirk et al (1992), and
Sharp (1997), the square data is transformed into circle data.

For experiment 2, since the circumference of the wooden circle was somewhat larger
than the circumference of the squares, a further size transformation was applied to the
data, using the same principle as the topological transformation. The circle-
transformed-into-a-square was reduced to 96% of its size, to provide a more exact
comparison of the two shapes. The figure 96% was a (very reliable) average of semi-
randomly-inspected vertex files of transformed circles and squares in Experiment 2.

An important point is that the firing rates in the circle are taken from the 96%-circle-
transformed-into-a-square firing rate maps. This is the fairest procedure. Both the
square and the 96%-circle-transformed-into-a-square have exactly the same
smoothing as a fraction of their total area (because they now have the same area). On
the whole, the procedure tended to reduce the peak rate of the fields in the circle.

Data analysis - Indices of similarity of firing within and across shapes

This section describes the quantitative measures or indices used to give an idea of
how similar or different the place cell firing patterns across shapes are. It is first
necessary to describe why these measures are used. When firing patterns are similar, a
cell firing, say, in the north west region of a square, fires in the north-west region of a
circle. We can call such a firing pattern “homotopic”. When firing patterns are different, this can occur in two basic ways. The extremes are considered first. A given cell which fires in one environment may be silent in another. This pattern might be described as “monotopic”, meaning that the cell essentially only fires in one of the shapes. Alternatively, a cell which fires in the north-west region of a square may fire, at a similar rate, in the centre of a circle. This pattern can be described as “heterotopic”. Obviously intermediate patterns based on these extremes are possible. A homotopic cell may fire at consistently lower rate in one shape, without actually falling below the 1.0 Hz threshold in that shape. A cell may be both heterotopic and show consistent peak rate differences. The important thing to note is remapping may be seen in both field peak rate differences and field peak position differences. The analysis used needs to attend to both of these kinds of differences. Broadly speaking, when remapping occurs we would expect that the across-shape differences in rate and position are larger than the within-shape differences. If remapping increases with experience, we expect that these differences increase with time.

Several measures or indices of the similarity of place field patterns within and across shapes are used in the analysis in the present thesis, as appropriate. These measures are described in this section. All these measures are based on the place field peaks after smoothing, derived as previously described in the section “derivation of place field characteristics”. The analysis focuses on two aspects of the place field peak: the rate of the peak (Hz) and the position of the peak (x, y coordinates, units in pixels).

For each cell, over all the available trials on a given day, the following measures are calculated from the firing rate maps.
Rate-related measures - all the trials contribute data

1) The average trial-to-trial within-shape rate difference.
   For instance, if a cell’s peak rate is 2.0 and 3.0 Hz in two trials of a square, and 5.0 and 6.0 Hz in two trials of a circle, the average trial-to-trial within-shape rate difference is the average of 1 and 1 Hz, ie. 1 Hz.

2) The average trial-to-trial across-shape rate difference
   In the above example, the average across-shape rate difference is the average of 2, 3, 3, and 4 Hz, ie. 3 Hz.

3) The average trial-to-trial within-shape rate difference/average trial-to-trial across-shape rate difference ratio. This is commonly abbreviated as the Rate w/a ratio.
   Continuing with the same example, the Rate w/a ratio would be 1 Hz/3 Hz, ie. 0.33.

Note that all the trials contribute data to these measures.

Distance-between-peak related measures - not all trials necessarily used

For these measures, the x, y position of a “field” below 1.0 Hz is ignored. Euclidean distances are calculated.

1) The average trial-to-trial within-shape distance-between-peaks (DBP).
   If a cell’s field peak is (x first, y second) 100, 100 and 100, 108 in the two square trials, and 100, 200 and 100, 208 in the two circle trials, the average trial-to-trial within shape distance-between-peaks (DBP) is 8 pixels.

2) The average trial-to-trial across-shape DBP
   In the above example, the average trial-to-trial across shape DBP is the average of 100, 108, 92, and 100 pixels ie. 100 pixels.
3) The average trial-to-trial within-shape DBP/average trial-to-trial across-shape DBP ratio. This is commonly abbreviated as the DBP w/a ratio. Continuing with the example given, the DBP w/a ratio would be 8 pixels/100 pixels, ie. 0.08.

Note that these measures examining peak position are not symmetrical to those examining rate issues. Basically, the peak position measures are based on subsets only of the available cells. The rate measures are based on the entire sample of recorded cells. Clearly, if a cell only fires at or above the 1.0 Hz threshold in one shape, there cannot be an across-shape DBP. Similarly, if a cell fires only twice, once in each shape (a situation much less frequent), then there cannot be a within-shape DBP. Thus, for a cell to contribute to the Within/Across DBF ratio, it must, at minimum fire above threshold in two trials in one shape, and in one trial in another shape.

It is important to note a simple fact about averaging over a group of cells using the absolute Within and Across values. The mean Within/Across ratio values for a group of cells cannot simply be surmised by dividing the mean absolute Within values by the mean absolute Across values. This is so whenever there are more values feeding into one average than another. For instance, when all cells fire in four trials, there are only 2 Within-shape values, but there are 4 Across-shape values, as the examples above illustrate. Note further that in situations of remapping, it commonly occurs that some cells have only within-shape datapoints for peak position measures. In other words, it is to be expected that cells which fire in one shape only cannot contribute to any measures requiring across-shape peak position data.
The strengths and weaknesses of the measures are discussed later in relation to concrete data. Because the w/a DBF ratio measure is not completely adequate for our purposes, another measure related to the firing across shapes is also used where appropriate. This is described in the next section. Here, all that needs to be said in summary is that when patterns are similar, we expect w/a ratios to be around 1.0, and when patterns are divergent, we expect w/a ratios between 1 and 0. If remapping increases with experience, we expect the w/a ratios to decline over days.

**Centroid of peaks comparison measure (CPCP distance)**

This is another measure of how far apart the field peaks are in one shape versus another shape, but it is not as sensitive to trial-to-trial differences. It averages over these trial-to-trial differences. In this comparison the euclidean distance from the centroid of the field peaks (in shape 1) to the centroid of the field peaks (shape 2) is calculated. This distance is abbreviated as the CPCP distance. If a cell’s peak is 100, 100 and 100, 108, and 100, 116 in the square the centroid of its field peaks in the square is 100, 108. If a cell’s peak is 100, 200 and 100, 208, and 100, 216 in the circle, the centroid of its field peaks in the circle is 100, 208. The CPCP distance is 100 pixels. This measure should not be confused with the average Across-shape DBF measure, which reports trial-to-trial differences in peaks.

As the maximum number of trials per shape per day is very low (2 or 3), and the CPCP measure is calculated daily, this measure is very unlikely to produce falsely small distances between centroids in the centre of the environment from noisy firing all over the environment. This is in addition to the fact that place cells simply do not fire in this way. The CPCP measure is simply designed to factor out fluctuations in
place field peaks associated with limited sampling, particularly for larger fields for instance, and fields at the edge of the environment (both linear and smaller circular fields - see eg. Muller, 1996). Obviously, homotopic patterns will tend to produce low CPCP distances, heterotopic patterns will tend to produce high CPCP distances. A reasonable question is: how low and how high? The next section describes a model or simulation which offers a quantitative benchmark by which to calibrate at least part of the answer to this question. When complete remapping occurs, it is commonly said, as we have seen in Chapter 4, that where a cell fires in two environments, knowledge of its field in one environment cannot be used to predict its field in another. This is like saying that the relationship between the fields in the two environments is random. This random relationship was modelled with a Monte Carlo simulation.

**Monte Carlo simulation of the distribution of peaks assuming random firing**

A Monte Carlo simulation of peak firing was performed, to test how likely it is that field peaks are close together by chance. In the analysis used for this study, the size and shape of a field is ignored. In a sense, the field is considered as a single point. Since this is so we can model what a random distribution of field peaks would look like in a relatively straightforward manner. The assumptions are made that field peaks may occur with equal probability throughout the extent of an environment, and that the topological transformation of circle data into square data does not affect this flat probability distribution.

Consider two squares of the same size. One square represents the circle-transformed-into-a-square, the other represents the square. Because the shapes share the same laboratory space, the two modelled squares share the same Cartesian coordinate grid.
The simulation can thus simply randomly generate pairs of x, y coordinates, and calculate the euclidean distance between each pair of x, y coordinates. Each coordinate pair represents one cell firing in two different shapes. On the basis of vertex files, the first simulation used a 187x187 pixel grid, to model the environments of experiment 1, while the 2nd simulation used a 200x200 pixel grid, to model the environments of experiment 2. The grid size used for experiment 1 is at the lower end of the range of observed grids, making it harder for a cell to have close across-shape peaks.

10,000 coordinate pairs, or cells, were created, and frequency distributions of the distances between peaks were generated using a standard statistical package (SPSS). These distributions can be used to compare the observed sample of cells to a random sample. They can also be used to derive a cutoff distance between peaks for a single cell, less than or equal to which a cell is considered to be firing in a similar position in both environments (a “homotopic” pattern), for a given probability level. A 5% probability level was used. Thus a statement can be made that there is only a 5% chance that, if firing were random, a cell’s field peaks in the two environments would be less than or equal to x pixels apart.

The simulation clearly does not take account of factors affecting reproducibility of field peaks, but may be considered to model the field peak for infinite position sampling in a discrete time period, with no measurement errors of any sort. In my view, the simulation is best compared with the measure that is not sensitive to trial-to-trial differences but averages them out, ie. the CPCP measure, which compares the
centroid of the peaks in the square with the centroid of the peaks in the circle-transformed-into-a-square.

**Histology and cell localisation**

After completion of the experiments, each rat was killed with an overdose of sodium pentobarbital (Lethobarb, 10mg) and perfused transcardially with saline followed by 4% paraformaldehyde. The brain was extracted and stored in 4% paraformaldehyde, and later sliced coronally into 40 μm thick sections, which were mounted and Cresyl-Violet Nissl-stained to aid visualisation of the electrode track and tip relative to cell bodies. As described in the Chapter 3 on hippocampal physiology, there are various electrophysiological markers of where the electrodes are in the hippocampus, and these were also used.
CHAPTER 7:  
THE PILOT STUDY

Introduction

In this exploratory study, rats were recorded in two different environments, within the same room, which were intentionally different in several ways. On the basis of studies by Muller and Kubie (1987), Quirk et al, (1992), and Sharp (1997) it was thought that the rats might show remapping across the two environments: then, importantly, further trials would elucidate the nature of the firing patterns, and determine on what basis the animals remapped if they did so, and how important shape was in this process. Most of the implications of the results were formally tested in experiments 1 and 2. However, one aspect of the results - remapping based on different room positions - was not really further explored in the experiments that followed, and some attention will be paid to this result.

Methods

There were 7 subjects in the pilot experiment: r961, r962, r974, r956, r975, r966 and r977. Figure 7 shows the laboratory set up used in this experiment, which was conducted in a different room from that used in experiments 1 and 2. Figure 7 suggests the ways in which the environments were different. One environment was a morph box configured as a square in position A. The other environment was a morph box configured as a circle in position B. Lighting conditions were different in the two environments. The paper on the floor on which the rats ran was generally not changed, and thus local cues could serve to differentiate the environments. Where animals did show patterns suggestive of remapping, probe trials were conducted to
Distances and measurements

Ax to Bx:
A’s centre is 90cm west and 30 cm south of B’s centre

A = Square with sides 62cm x 62cm
B = Circle 76cm in diameter

Figure 7. Diagram of layout of the Pilot experiment.

Dark and light shading in the square and circle indicate (roughly) the amount of incident light in these environments.
examine the basis of the differential patterns between the morph square and morph circle.

Results
In all 7 animals were run in the two environments. For some animals, only a few place cells were recorded (sometimes only 1 or 2 cells simultaneously). In 4 animals, surprisingly, there was little clear evidence of remapping. Most cells did not have obviously different fields in both environments. These animals had had little in the way of training, and were not run for long.

Clear evidence of remapping was obtained from three animals. As others had found, there was evidence of both monotopy (a cell firing in one environment only) and heterotopy (a cell firing in different positions in the two environments). For one of the animals (r974), where initial exposures to the environments were recorded, there was some limited evidence that for initial exposures, the majority of cells were similar across environments, and that increased experience increased remapping patterns.

Two other animals (r966 and r977) showed very obvious evidence of remapping, and rather more cells were recorded from these animals, making the results easy to interpret. (Because these showed clear signs of remapping, these two animals were used again in experiment 1B.)

Animal r966 was trained before it was implanted with electrodes. Animal r966 had received a great deal of training, sporadically over more than a month, in the two environments, and I thought that this might be important in its production of
remapping firing field patterns. There was also evidence from one cell of the
evolution of a remapping pattern. However, animal r977 showed remapping patterns
as soon as testing began, after a very short one day training period.

On three animals, r966, r974, and r977, probe trials were conducted beyond that
applied to most of the other animals, where, for instance, the floor paper might be
changed. (Incidentally, this generally had little effect, despite the fact that the paper
was sometimes unchanged for several days, and thus allowed to accumulate location-
specific markers.) These probe trials included the following procedures:

1) Morph Box rotations.
2) Translations.
3) Changing the shape of the environments, such that the position in the lab usually
occupied by the morph square (Position A in Figure 7) was occupied by a morph
circle, and the position usually occupied by the morph circle (Position B) was
occupied by a morph square.

Morph Box rotations had no obvious effect on the firing patterns. Small translations
of 15-20 cm had no obvious effect on the firing patterns.

Figure 7a shows the effect of changing the morph shapes in both Position A and
Position B. The Figure is designed in matrix-style, and shows firing rate maps from
two place cells from r966, which are representative of the results obtained from r966,
r977, and r974. For each cell, four firing rate maps represent all the possible four
combinations of two positions (A and B) and two shapes (square and circle). For each
cell, two firing rate maps show the standard testing configurations marked
Position-based, rather than shape-based, remapping

<table>
<thead>
<tr>
<th>Enclosure shape</th>
<th>Cell 1</th>
<th>Enclosure shape</th>
<th>Cell 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Position in room</td>
<td></td>
<td>Position in room</td>
</tr>
<tr>
<td></td>
<td>Position A</td>
<td></td>
<td>Position A</td>
</tr>
<tr>
<td></td>
<td><strong>Probe</strong></td>
<td></td>
<td><strong>Probe</strong></td>
</tr>
<tr>
<td>as Circle</td>
<td>2.1 Hz Standard</td>
<td>6.5 Hz Standard</td>
<td>0.0 Hz <strong>Probe</strong></td>
</tr>
<tr>
<td></td>
<td>2.5 Hz <strong>Probe</strong></td>
<td></td>
<td>4.1 Hz <strong>Probe</strong></td>
</tr>
<tr>
<td>as Square</td>
<td>2.9 Hz <strong>Probe</strong></td>
<td></td>
<td>0.0 Hz <strong>Probe</strong></td>
</tr>
</tbody>
</table>

Figure 7.a Matrix diagrams indicating that position influenced firing patterns more than shape.

The firing rate maps for two representative place cells in each of the four combinations of position and shape. Cell 1 is top, Cell 2 bottom. It is clear that position, not shape, is the key determinant of the difference in firing patterns between the standard configurations. To appreciate this point at a glance, simply note that the similarity of patterns is vertical through each matrix diagram, and not horizontal.
The influence of position and shape over locational firing

Figure 7b. Upper diagram (a) shows steps in a further probe trial series examining effects of translation. This probe trial series was conducted two days after the trials shown in the previous figure, and with the same place cells. The circle in position B was translated, in steps, southwards, and westwards, to position A. In position A, the circle was made into a square. Finally, the same enclosure was placed in position B as a circle, as in the standard configuration. In order to present the steps clearly, the distance between position A and B has been exaggerated.

Lower diagram (b) shows simple firing maps of cell 1 (see previous figure) depicting where the cell fired in the environments. Fields are shown simply as clusters of green pixels in order to bring out subfields.
“Standard”, and two rate maps show the probe configurations marked “Probe**”. The easiest way to appreciate this Figure is to note that similarity of the fields is vertical down the page, while the dissimilarity is horizontal across the page. In other words, it is primarily position, and not shape, that is responsible for the remapping. No further study was made of what cues might be responsible for the rats’s sense of the different positions.

Saying that position was primarily responsible for the remapping seen is accurate. This is not, however, to say that shape made no difference at all. In Figure 7a, it can be seen that there are three small fields in the circle in position A, and only two in the square in position A. The subfield (light blue) in the north of the circle in position A varied in strength compared to the field in the south-east region. Nevertheless, even in this cell, two fields are very similarly located in both environments: one (the most obvious) in the extreme south-east, and one just north and west of the south-east field.

As a further test of the idea that it was primarily position, and not shape, that was the basis of the remapping, a further probe trial series was performed, for r966 only, two days after the trials shown in Figure 7a. This further probe trial series is shown in Figure 7b. The cell shown in this figure is the same as “cell 1” in Figure 7a. To bring out the subfields which can be obscured by the standard normalization procedure for showing fields, fields (shown in part B of the Figure 7b) are simply represented as green pixel clusters. Part A of Figure 7b shows shows the steps of the translations, which were done in 15-20cm steps in a given direction. Step 1 represents the first translation, both westwards, and southwards from the standard configuration (Position B). The first standard trial in the morph circle in position B is not shown. Steps 2 and
3 were further westward translations. Step 4 was a westward and southwards translation. In order to present the steps clearly, the distance between position A and position B has been exaggerated in Figure 7b. For step 5, the morph circle was configured as a morph square. Finally, for step 6, the same morph box was reconfigured as a circle and placed back in the standard position for the morph circle (position B). Recordings were made at each step in the series.

Part B of Figure 7b shows the fields recorded from the cell, simply represented as green clusters. There are some subtleties, but I wish to draw attention to two obvious features. First, the fields in the standard configurations look similar to those obtained two days earlier (see Figure 7a, cell 1: Square in position A and Circle in position B). Second, and most importantly, fields and subfields vary greatly while the shape is held constant (circle: trials 1, 2, 3, 4, 6), so it is obvious that room position is a key determinant of firing. Phrased differently, the cell shows remapping patterns while in the same shape. The implications of the results of changing morph shape in the same position (Figure 7a) are further underlined by those of changing position while keeping shape constant (Figure 7b).

Summary and the implications for further experiments

The pilot study suggested various ideas worth pursuing:

1) That during early trials the firing patterns in the morph box, the firing patterns would be similar across the shapes, if the shapes were in the same position in the laboratory. This was clearly contrary to the expectations of most workers in the hippocampal place cell field.

2) That experience might be an important factor in remapping.
The pilot study thus suggested it was worth doing a simple standardised and quantitative study in which all else but shape would be held constant, in a simple laboratory testing space with relatively few cues. It seemed useful to have one experiment where training in one shape would be minimal, before exposure to the second, but many cells would be recorded, in order to fully test the assumption of obligatory remapping for circle and square walled environments. This became Experiment 1A. It also seemed useful do an experiment which extended the training period in the first shape, before exposure to the second, and particularly, extended the testing period with several repeated exposures to the two shapes. Here, the focus was on getting cell-stability in order to follow individual cells over time. This became Experiment 1B.

As described in the next chapter, at the end of the 6 day repeated-exposures period of Experiment 1B, cells were still not close to showing fully-remapped patterns. One animal had to be killed on day 3, and good one-week stability was not obtained for another animal. Accordingly, it was necessary to perform a similar experiment to experiment 1B, but recording for a much longer time where necessary, and making sure good stability was obtained for at least some extended periods in each animal. This became the repeated exposure time-series part of Experiment 2.

Animals r966 and r977 were used again in experiment 1B

Animals r966 and r977 were used in experiment 1B (Chapter 8). This was to anticipate possible criticisms that remapping might not be observed in experiment 1B because its subjects belonged to a group of rats somehow especially inattentive to the
environment. Since experiment 1 involved the use of a single morph box, configured as a square and circle on alternate trials, it is important to state that the degree of exposure these two animals had in the morph circle and morph square in the pilot study was considerable. This extensive experience is described below.

In all, including training, testing, and probe phases, r966 experienced 571 minutes in the morph square, and 743 minutes in the morph circle. This experience was gained over a 73 day period. There was an interval of 72 days between r966's last experience in the pilot experiment room, and his first day of training in the morph square in experiment 1B.

In all, including training, testing, and probe phases, r977 experienced 272 minutes in the morph square, and 220 minutes in the morph circle. This experience was gained over a 10 day period. There was an interval of 55 days between r977's last experience in the pilot experiment room, and his first day of training in the morph square in experiment 1B.
CHAPTER 8:

EXPERIMENT 1A AND EXPERIMENT 1B

Experiment 1: Aims, focus of analysis, terminology

The main aim of experiment 1 was to test the idea preliminarily suggested by the pilot study that upon initial exposures to the morph box, there would not be remapping across the morph square and the morph circle.

Reference will be made throughout this chapter to the “main experiment” of experiment 1A, and experiment 1B. This term applies to day 1 of Experiment 1A, and days 1 to 6 of experiment 1B. Only the results of day 1 in each experiment were subjected to formal, quantitative analysis. The focus of this analysis is to test the specific hypothesis that rat place cells would not show shape-based remapping on day 1 of the experiments. Other data is presented descriptively only, where appropriate.

Methods for Experiment 1A and 1B

Common procedures to Experiment 1A and 1B

The laboratory setup was the same for experiments 1A and 1B, and is shown in Figure 8. Important features were the use of black curtains to seal off the testing environments from the laboratory room (Muller and Kubie, 1987; Bostock et al, 1991) and the use of a large white cue card suspended on one side (lab east) of the curtain environment, to provide a stable polarising cue. This cue card measured 102cm in height, 77cm in width, and was suspended from an inner rail 25cm inwards of the black curtain rail. The top of the cue card was 195cm from the floor. For ease of reference this large external card will sometimes be called the Jeffery cue card.
Layout of Experiment 1a and 1b

A single morph box is used to create square and circle shaped walled enclosures in the same position in the testing space.

Figure 8. Diagram of laboratory layout used in experiments 1a and 1b
(Jeffery and O'Keefe, 1999). As in the pilot experiment, a holding platform was used, where the rats were placed between trials. Thus, rats were not taken out of the lab room between trials. In summary then, rats were taken out of the curtained environment between trials.

A small point is that the morph box has a small vertical seam where the two ends of the walls meet. The morph box was always placed such that this seam was on the laboratory west side of the box.

**Experiment 1 - Morph 'diamond' probe phase**

One or more animals in each group were run in trials where the morph box was configured as a square with a different orientation. The morph square was rotated 45 degrees from its usual position. As always, this environment had the same centre as the morph square and morph circle. This configuration is referred to, for simplicity, as the morph diamond. These trials were standardised in so much as they were always performed on the day after the end of the main experiments in 1a and 1b (thus on day 2 in experiment 1a, and on day 7 in experiment 1b).

**r1062: Delay test**

As a control for the variable of time without further environmental experience, r1062 from experiment 1b, which showed highly similar patterns across the morph circle and morph square on Day 6 of experiment 1b, was also delay tested in its original setup, the morph square and morph circle. Delay for r1062: 32 days since last exposure to curtained environment. (On the day previous to the delay test, the animal had been brought into the laboratory room for an hour and a half. If anything, such a
<table>
<thead>
<tr>
<th>Animal</th>
<th>Number of cells</th>
<th>No. of active tetrodes</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rl004</td>
<td>32</td>
<td>5</td>
<td>CA1</td>
</tr>
<tr>
<td>rl020</td>
<td>12</td>
<td>5</td>
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</tr>
<tr>
<td>rl039</td>
<td>2</td>
<td>1</td>
<td>CA1</td>
</tr>
<tr>
<td>Experiment 1B</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>r995</td>
<td>2</td>
<td>1</td>
<td>CA1</td>
</tr>
<tr>
<td>r977</td>
<td>4</td>
<td>1</td>
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</tr>
<tr>
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<td>1</td>
<td>CA3</td>
</tr>
<tr>
<td>rl062</td>
<td>10</td>
<td>1</td>
<td>CA1</td>
</tr>
</tbody>
</table>

Table 8.1 Table documenting number and anatomical location of cells contributing to the analysis of day 1 of Experiment 1A and day 1 of Experiment 1B.
procedure should help retrieve expectations of intra-curtain environments, and thus aid any “remapping”.

Results of the Monte Carlo Simulation for Experiment 1

Frequency distribution graphs and tables for the distance between the two x, y coordinates of 10,000 cells are obtained, to evaluate the probability distribution of place field peaks being close together by chance in a grid size of 187x187 pixels.

The mean distance between the “cells” was 97 pixels (0.5 pixels sem). To determine the classification of a single cell, a 5% level of probability was used to produce a cut off distance between the peaks in the two environments: this was located between 23 and 24 pixels. Thus 23 pixels was taken as the distance-between-peaks less than or equal to which a cell might be described as homotopic. Complexities such as allowance for spatial resolution, and experimental error in placing shapes in exactly the same place on subsequent trials, were ignored in the model. The real size of the morph square is about 62 x 62 cm, or 205 x 205 pixels. Since the hypothesis to be tested was that place field peaks in each shape would be located close together after transformation, it was thought appropriate to err on the side of stringency.

Results

46 place cells contributed to the analysis of experiment 1A, and 20 cells contributed to the analysis of experiment 1B. Table 8.1 gives a breakdown of the number of cells recorded from each animal, and their location in the hippocampus based on histological analysis, and electrophysiological criteria. There was no ambiguity about
the anatomical locations of any of the cells. All cells were recorded from the CA1 layer, except the cells from animal r966 in experiment 1B, which were recorded from CA3. No differences could be seen in these cells as compared to those from CA1, and the analysis grouped them all together.

**Experiment 1A - Day 1 - Descriptive overview**

The subjects in experiment la were rats r1004, r1020, and r1039. After a single 24-minute training trial in the morph square, rats were run for four further test trials, two in each shape (alternating, beginning with the circle). 46 cells were recorded from 3 rats (see table 8.1).

Figure 8a shows the firing rate maps from the first circle test trial (not transformed), and the first square test trial, from all the 46 cells. As judged by eye, the firing rate maps across the two shapes were similar in all the animals. It can be seen that the firing rate maps of rat 1020 are somewhat messier than those of the other two animals. This may in part be attributed to uneven and slow coverage of the environment. 24 minutes on one day does not represent a great deal of training. Some idea of the poorer coverage by this rat can be gained by observing that there are more unvisited areas in the environments (denoted by white spots in the firing rate maps). Nevertheless, the similarities of the fields between the two shapes are much more obvious than the differences between them. Unfortunately, only two cells were recorded from animal r1039 (bottom right of Figure 8a), but again it is clear that the firing is similar across shapes in both cells.
Figure 8a
Firing rate maps from 1st and 2nd trials of day 1 for all 46 cells of Experiment 1A.
Figure 8b
See opposite for legend.
Figure 8b (see opposite page.)

Figure 8b on the facing page shows firing rate maps from all 36 cells recorded from animal r1004 in the first two trials of day 1 of experiment 1A.

There are 18 cells in each block. The left hand column in each block shows the firing rate maps in the circle. The middle column shows the firing rate maps for the circle-transformed-into-a-square. The right hand column shows the firing rate maps for the square.

All the firing rate maps in the circle and square have already been shown in Figure 8a.
36 place cells were recorded from rl004, and Figure 8b shows the firing rate maps for all 36 cells in the circle, transformed circle, and square respectively, again from the first two trials. Figure 8b gives a visual idea of the effect of the transformation. Note how the field peak rates are attenuated in the circle-transformed-into-a-square.

It is also clear that the rates between the shapes are rather similar for many cells. This is interesting, given the high variability in place cell firing rates (Fenton and Muller, 1998).

For two cells (Tetrode 1, cell 3, and Tetrode 7, cell 3), the field peak rate falls below 1Hz in the transformed circle, but it is notable that the fields in the untransformed circle and square appear to be homotopic.

In summary, judgement of the firing rate maps by eye would suggest that the fields are generally similar across shapes, and that the animals are not “remapping”.

**Experiment 1B - Day 1 - Descriptive Overview**

**Procedure**

The subjects in experiment 1b were rats r966, r977, r995, and r1062. After a training period of 8 to 10 trials in the square only over 3 days, rats were given six test trials a day for six days, three in each shape (alternating, beginning with the square). (Only two full days of data were obtained from r966, whose implant came off on day 3 of the experiment). Only day 1 of the experiment, when the rats were first exposed to the circle, is described fully. 20 cells were recorded from 4 rats. Again, as judged by eye,
Figure 8c. Firing rate maps of all 20 cells for the middle two trials on Day 1 of Experiment 1B.

As with day 1 of experiment 1A, the pattern of firing is very similar across shapes. The pattern of firing of the four cells of r966, recorded from the CA3 region, are indistinguishable from the other firing patterns.

The small circle shown for cell 7 of r966 is the firing rate map from another trial.
<table>
<thead>
<tr>
<th>Cell</th>
<th>Morph Circle</th>
<th>Morph Circle Transformed</th>
<th>Morph Square</th>
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<tr>
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<td>4.5</td>
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</tr>
</tbody>
</table>

Figure 8d. Similar (non-remapping) place field patterns, across the morph circle and morph square. Firing rate maps from 10 simultaneously recorded place cells, all from r1062 on day 1.
the firing rate maps across the two shapes were similar in all the animals. Figure 8c shows the firing rate maps from the middle two trials for all 20 cells on day 1. The similarity of the fields between the two shapes is very striking. Cell 7 of r966 did not have a field peak at 1 Hz or above in the circle trial depicted, but did so on another trial (shown top-right of the appropriate firing rate map).

Comparing Figure 8c and Figure 8a suggests that the actual place fields of the cells recorded on day 1 of experiment 1B are generally smaller than those derived from the rats of experiment 1A, and are, overall, more similar across shapes. This result may owe, at least in part, to better coverage of the environments, and/or to the longer training period of Experiment 1B. Note that there are not many white spots in the firing rate maps in Figure 8c, as compared to Figure 8a.

Another difference between the field patterns of Experiment 1A and 1B suggested by comparing Figures 8a and 8c, is that the fields in the sample from Experiment 1B are generally located closer to the edges of the environment. (This trend is not quantified in the sections below, but is mentioned in Discussion with relation to the Monte Carlo simulation of field peaks.)

Figure 8d is analogous to Figure 8b, and shows firing rate maps from the animal who contributed most cells to the data (r1062: 10 cells) including the firing field maps for the circle-transformed-into-a-square. (The square trial in Figure 8d is the one immediately after the trials shown in Figure 8c, and the circle trial is the same one shown in Figure 8c.) As a whole, the similarity of the fields and firing rates is remarkable.
In summary, the firing rate maps in the circle and square trials show very obvious similarities. There is surely no coherent basis after visual inspection of these maps for suggesting that the experimental hypothesis is falsified.

**Experiment 1A and 1B - Day 1 - Quantitative Procedures and Results**

In this section, the results of the various indices of similarity of firing are presented.

**Quantitative Procedures**

For each cell, the within and across Rate difference and DBP measures were calculated as described in the Methods chapter. These were as follows:

1) The average trial-to-trial within-shape rate difference.

2) The average trial-to-trial across-shape rate difference

3) The average trial-to-trial within-shape rate difference/average trial-to-trial across-shape rate difference *ratio*.

Measures 1 to 3 relate to field peak rate differences.

4) The average trial-to-trial within-shape distance-between-peaks (DBP).

5) The average trial-to-trial across-shape DBP

6) The average trial-to-trial within-shape DBP/average trial-to-trial across-shape DBP *ratio*.

7) The Centroid of peaks comparison measure (CPCP distance), calculating the euclidean distance from the centroid of the field peaks (in shape 1) to the centroid of the field peaks (shape 2) is calculated.

Measures 4 to 7 relate to field peak position differences.
In experiment 1A, these measures are based on four trials, two trials in each shape. In experiment 1B, these measures are based on six trials, three trials in each shape. The reader is reminded that the Within and Across measures are averages of the trial-to-trial differences. Only the CPCP distance measure factors out the trial-to-trial differences.

Statistics

Descriptive statistics were derived for measures 1 to 7. Linear correlation tests were performed on the Within vs Across measures for position differences and rate differences. Each cell contributed one datapoint. Pairwise cell-by-cell t tests were performed on the within shape measures vs the across shape measures. Linear correlations were also performed on the Rate and DBP w/a ratios. This test does not test for remapping or not remapping, but was done to see if cells show similar values on the similar/different dimension for both rate and peak position.

Quick Results summary for both experiments

For a quick comparison, Table 8.2 and Figure 8e summarise the quantitative and statistical results for Experiment 1A; Table 8.3 and Figure 8f do the same for Experiment 1B. F values, degrees of freedom, and t values are all reported fully in the tables.

Experiment 1A - Day 1 - Quantitative Results - See Table 8.2 and Figure 8e

One of the 46 place cells recorded did not have Within-shape DBP datapoints. This cell cannot contribute to the Within-Shape DBP to Across-Shape DBP correlations,
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<td>DESCRIPTIVES</td>
<td>CORRELATION</td>
<td>PAIRWISE t TEST</td>
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<tr>
<td>A) Field Peak position differences</td>
<td>Within shape DBP: Mean = 29.1 pixels, sem = 3.0 pixels, Across shape DBP: Mean = 29.4 pixels, sem = 2.3 pixels</td>
<td>r= 0.70, F (1, 43) = 41.36, p&lt;0.0001</td>
<td>df = 44, t = 0.143, p&gt;0.89</td>
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<td>Within shape DBP (n = 45), &amp; Across shape DBP (n = 45)</td>
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<tr>
<td>B) Rate differences</td>
<td>Within shape rate diff: Mean = 2.02 Hz, sem = 0.25 Hz Across shape: Mean = 2.19 Hz, sem = 0.23 Hz</td>
<td>r= 0.76, F (1, 44) = 59.23, p&lt;0.0001</td>
<td>df = 45, t = 0.992, p&gt;0.32</td>
</tr>
<tr>
<td>Within shape rate difference (n = 46) &amp; Across shape rate difference (n = 46)</td>
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<tr>
<td>C) Within/Across (W/A) ratios</td>
<td>Rate w/a ratio: cell mean = 0.95, sem = 0.06 DBP w/a ratio: cell mean = 1.02 sem = 0.06</td>
<td>No significant correlation, p&gt;0.61</td>
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<tr>
<td>Rate W/A ratio (n= 46) &amp; DBP W/A ratio (n = 45)</td>
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<tr>
<td>D) CPCP measure &amp; Homotopic cells</td>
<td>CPCP distance: mean = 21 pixels sem = 2.2 pixels</td>
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<tr>
<td>Assuming 23 pixel cut off distance: 30/46 cells are homotopic (65%)</td>
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<tr>
<td>Data for other cut off distances: &lt;=21 pixels: 27/46 (59%) &lt;=25 pixels: 32/46 (70%) &lt;=27 pixels: 34/46 (74%)</td>
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Table 8.2 Statistical results table, presenting various statistics associated with a lack of "remapping" in the animals of experiment 1A. All tests are two-tailed. "Sem" = Standard Error of the mean. "DBP" is an abbreviation for Distance-between-Peaks.

One of the 46 place cells did not have both Within-DBP and Across-DBP datapoints, and has been excluded from the Analyses in A) and C) part II.
Experiment 1A - No "remapping"

1) Within shape peak distance differences vs Across shape peak distance differences

\[ r = 0.70, p < 0.0001 \]

2) Within shape rate differences vs Across shape rate differences

\[ r = 0.76, p < 0.0001 \]

C) Rate Within/Across ratio vs Peak Distance Within/Across ratio

No significant correlation (p > 0.61)

Figure 8e. Scatter plots depicting relationships between variables within and across shapes for 46 place cells of Experiment 1A. One cell which does not have a within-shape distance-between-peaks data point is excluded from the scatter plots of A) and C). Note that the axes in the first two graphs are not equal.
and was also excluded from the Across-Shape DBP descriptive statistics. It is included in the CPCP distance index.

The mean Within Shape DBP was 29.1 pixels (3.0 pixels sem). The mean Across Shape DBP was 29.4 pixels (2.3 pixels sem). The values are clearly very similar. The linear correlation for the two variables (see scatter plot A in Figure 8e) was \( r = 0.70 \), \( p < 0.0001 \). This suggests that cells with large within-shape DBPs also had large across-shape DBPs, and so on. Such a pattern might well be expected from cells with large fields, or cells that have generally lower reproducibility of firing. A pairwise t test was performed on these two DBP variables on a cell by cell basis, revealing no significant difference (\( p > 0.89 \)). The mean Within/Across (W/A) DBP ratio for the cells was 1.02 (0.06 sem). (Note that the W/A DBP ratio cannot simply be obtained by dividing the mean within shape DBP by the mean across shape DBP.)

The CPCP distance mean (based on all 46 cells) was 21 pixels. From the Monte Carlo simulation cumulative frequency chart (not shown), the probability of a single cell’s peaks being this close to each other is 0.042, assuming random firing. Since there are 46 cells, it is unnecessary to work out in detail, whether applying the central limit theorem or using the cumulative frequency table since the distribution is somewhat skewed, the vanishingly small probability that the sampled population is composed of cells with peaks that are not systematically related to each other. Using the 23 pixel cut off distance, 30 out of 46 cells (65%) may be described as homotopic. Table 8.2 gives the percentage of homotopic cells for other cut off points. The less stringent cutoff of 25 pixels (the cutoff distance in a 200x200 grid: 2\textsuperscript{nd} Monte Carlo simulation for experiment 2) produces a figure of 70% homotopic cells.
The mean within shape rate difference was 2.02 Hz (0.25 Hz sem). The mean across shape rate difference was 2.19 Hz (0.23 sem). The values are again similar. The linear correlation for these two variables (see scatterplot B in Figure 8e) was $r = 0.76$, $p < 0.0001$. This suggests that cells with relatively high rate differences across shapes showed this pattern simply because they were more variable generally. The pairwise cell-by-cell t test showed no significant differences ($p > 0.32$). The mean cell W/A rate ratio was 0.95 (sem 0.06), again close to unity.

Finally, a scatter plot was made of the w/a rate ratio for each cell against the w/a DBP ratio for each cell, for 45 cells (see Plot C in Figure 8e). There is no significant correlation ($p > 0.61$). This suggests that there is no tendency for cells which are similar in terms of peak rate to be similar in terms of peak position, and so on.

**Experiment 1B - Day 1 - Quantitative Results - See Table 8.3 and Figure 8f**

All 20 cells contributed to all measures.

The mean Within Shape DBP was 18.2 pixels (3.0 pixels sem). The mean Across Shape DBP was 21.8 pixels (2.5 pixels sem). The values are clearly very similar. It is notable that they are both smaller than the equivalent values in experiment 1A. The linear correlation for the two variables (see scatter plot A in Figure 8f) was $r = 0.74$, $p < 0.00025$. Again, this suggests that cells with large within-shape DBPs also had large across-shape DBPs, and so on. The pairwise t test for these variables revealed no significant difference ($p > 0.09$), though this probability is appreciably smaller than the equivalent statistic in experiment 1A. The mean Within/Across (W/A) DBP ratio for
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<th>PAIRWISE t TEST</th>
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<tbody>
<tr>
<td><strong>A) Field Peak</strong>&lt;br&gt;position differences</td>
<td><strong>Within</strong> shape DBP: Mean = 18.2 pixels, sem = 3.0 pixels, <strong>Across</strong> shape DBP: Mean = 21.8 pixels, sem = 2.5 pixels</td>
<td>$r = 0.74$, $F(1, 18) = 21.76$, $p &lt; 0.00025$</td>
<td>df = 19, $t = 1.745$, $p &gt; 0.09$</td>
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<tr>
<td><strong>B) Rate differences</strong>&lt;br&gt;</td>
<td><strong>Within</strong> shape rate diff: Mean = 1.97 Hz, sem = 0.30 Hz <strong>Across</strong> shape: Mean = 2.14 Hz, sem = 0.30 Hz</td>
<td>$r = 0.88$, $F(1, 18) = 59.37$, $p &lt; 0.0001$</td>
<td>df = 19, $t = 1.073$, $p &gt; 0.29$</td>
</tr>
<tr>
<td><strong>C) Within/Across (W/A) ratios</strong>&lt;br&gt;</td>
<td><strong>Rate</strong> w/a ratio: cell mean = 0.96, sem = 0.06 <strong>DBP</strong> w/a ratio: cell mean = 0.81 sem = 0.07</td>
<td>No significant correlation, $p &gt; 0.73$</td>
<td></td>
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<tr>
<td><strong>D) CPCP measure</strong>&lt;br&gt;&amp; Homotopic cells</td>
<td><strong>CPCP distance</strong>: mean = 14 pixels sem = 2.0 pixels</td>
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Table 8.3 Statistical results table, presenting various statistics associated with a lack of “remapping” in the animals of experiment 1B. All tests are two-tailed. “Sem” = Standard Error of the mean. “DBP” is an abbreviation for Distance-between-Peaks.
Experiment 1B - No "remapping"

A) Within shape peak distance differences Vs Across shape peak distance differences

$r = 0.74, p<0.00025$

B) Within shape rate differences Vs Across shape rate differences

$r = 0.88, p<0.0001$

C) Rate Within/Across ratio Vs Peak Distance Within/Across ratio

No significant correlation ($p>0.73$)

Figure 8f. Scatter plots depicting relationships between variables within and across shapes for 20 place cells of Experiment 1B. Note that the axes are not equal in plots A) and C).
the cells was 0.81 (0.06 sem). This is smaller than the W/A DBP ratio for experiment 1A (which was 1.02).

The CPCP distance mean was 14 pixels. From the Monte Carlo simulation cumulative frequency chart, the probability of a single cell's peaks being this close to each other is 2.0%, assuming random firing. Since there are 20 cells, it is again unnecessary to work out the precise value of the vanishingly small probability that the sampled population is composed of cells with peaks that are not systematically related to each other. Using the 23 pixel cut off distance, 18 out of 20 cells (90%) may be described as homotopic. The use of cut off values of 21, 25, and 27 pixels give the same result.

The mean within shape rate difference was 1.97 Hz (0.30 Hz sem). The mean across shape rate difference was 2.14 Hz (0.30 sem). The values are again similar. The linear correlation for these two variables (see scatterplot B in Figure 8f) was $r = 0.88$, $p<0.0001$. This suggests that cells with relatively high rate differences across shapes showed this pattern simply because they were more variable generally. The pairwise cell-by-cell t test showed no significant differences ($p>0.29$). The mean cell W/A rate ratio was 0.96 (sem 0.06), very close to unity.

The scatter plot made of the w/a rate ratio for each cell against the w/a DBP ratio for each cell (see Plot C in Figure 8f) shows no significant correlation ($p>0.73$).

**Experiment 1A and 1B - Day 1 - Discussion: Summary and Measures of similarity**

The results of experiment 1 on day 1 clearly confirm the experimental hypothesis that the cells do not show remapping, certainly not as a group. In particular, the results
indicate that after exposure to one shape (the square), the initial exposures to another shape (the circle) produce similar patterns of firing in place cells to those seen in the training shape, given that the shapes are in the same position in the laboratory testing space and that all efforts are made to maintain similarity in the rats’ direction sense. This was true both after brief exposure (Exp 1A: 24 minutes) and more extensive exposure (Exp 1B: 8-10 trials of eight minutes each over 3 days) to the square.

Before moving on to other parts of experiment 1, it is useful to consider the strengths and weaknesses of the various measures used. This will also prepare for the use of these measures in experiment 2. In my view the rate measures are not problematic. The CPCP distance has the advantage over the W/A DBP ratio in that it is more tangibly connected with reality, given that the size of the environments is known to the experimenter (and we can make models of the probability distributions of proximal peaks). A concrete measure of absolute proximity is provided. The absolute Across DBP also has this quality, but clearly the measure is meaningless as an index of similar firing unless one also has the Within DBP. The W/A DBP ratio has the advantage over the CPCP distance that it is precisely qualified by this within-shape reproducibility of peak firing. Thus if the fields are noisier or larger, if the LEDs give a less precise indication of the rat’s “cognitive center” (or at any rate a particular range of points on the rat’s head), or if the fields are just less stable (eg. if the environments are not so well traversed, and mapped with fine grain, initially), the w/a DBP ratio will not underestimate the similarity in the field peaks that the CPCP measure may. For instance, despite the fact that the fields in experiment 1A do look very similar, only 65% of the cells can be stringently categorised as homotopic. Yet the w/a DBP ratio over 45 of 46 cells is 1.02.
The w/a DBP ratio potentially has an additional problem when the field peaks across trials are located quite close together. It can produce what one might describe as misleading deviations from unity. Consider the following purely theoretical examples. One cell with a mean 9 pixel within, and 7 pixel across distance, produces a ratio of 1.29. Another cell with a mean 3 pixel within and 8 pixel across distance produces a ratio of 0.38. To take the second theoretical cell, we would not, at least preliminarily, consider that a cell with an average across distance of 8 pixels was indicative of the remapping that a ratio of 0.38 might imply. So the CPCP measure is also useful. In fact, however, these kinds of extreme results did not occur. It may be noted that despite having a significantly smaller CPCP mean of only 14 pixels, the w/a DBP ratio mean for the 20 cells of experiment 1B was clearly lower than that of experiment 1A (0.81 versus 1.02). There was one cell in Experiment 1B similar to the extreme theoretical examples mentioned, in that it had within/across values of 5.3 and 8.6 pixels, producing a w/a ratio of 0.62. Its CPCP distance was 8 pixels. Less extreme examples than this also contribute to this lower, 0.81, figure.

Finally, though it is not of great pertinence to experiment 1, because the CPCP measure only requires Across datapoints, it produces an index of similarity based on more of the available data. Use of the number of homotopic cells based on the CPCP measure and the Monte Carlo simulations, to be reliable, and in particular to feed into parametric statistics (as will be done for the combined data of experiment 2) does clearly require many cells. This condition is satisfied by 46, and 20 cells, in my view.
Figure 8g. Firing rate maps for 8 cells of animal r995 on day 4 of experiment 1B.

The firing rate maps are arranged in rough order of similarity of firing across shapes, from similar (top) to dissimilar (bottom). It appears that cells 5 to 8, and arguably cell 4, show patterns indicative of remapping.
Figure 8h Largely similar firing across shapes in the Morph Diamond Probe phase of Experiment 1.

Diagram shows firing rate maps for 14 cells, taken from animal r977 on day 7 (Experiment 1B). Trials shown are, from left to right, the 1st circle, 1st diamond, and 2nd square, trials of the day. No formal analysis has been performed on these trials, but it is clear that firing is "homotopic" across all shapes for most of the cells. Further, nearly all cells show similar firing patterns in the square and circle. Firing patterns suggestive of possible remapping in at least one shape include some cells shown in the right hand column (eg. 8, 9, 13, 14).

Note that the angular location of place fields in the morph diamond is not predicted by rotating their position in the morph square by 45 degrees (eg. consider cells 5, 6, 11, and 12). The rat's sense of direction appears to have been well "anchored" (see Methods.)
**Experiment 1A and 1B - Further trials - Descriptive Results**

Experiment 1B only - Days 2 to 6 of main experiment

The further time-series trials of days 2 to 6 of Experiment 1B may now be considered a kind of pilot series for experiment 2, which was explicitly designed to look at the variable of time/experience on shape-based remapping, and go for up to 21 days, a considerably longer period of time than experiment 1B. Preliminary evidence that cells remap with experience was seen in Experiment 1B.

This section presents examples of firing patterns from two rats, one showing strong evidence, the other showing little evidence, of across-shape divergent firing patterns after experience.

Figure 8g shows firing rate maps for 8 cells recorded from r995 on day 4 of the time-series. On visual inspection, half of the cells appear be in a remapped state, and one or two more cells show more subtle dissimilarities of firing. Figure 8g shows the *most dissimilar* across-pattern of firing on one day, from the whole sample of animals and days in the main experiment. Figure 8h shows firing rate maps for 14 cells from r977, at the other extreme, showing a great deal of similarity on day 7 of the experiment. No more than 2 or 3 cells show obviously dissimilar firing patterns across the square and circle. Figure 8h also takes us to the next section.

**Experiment 1A and 1B - Morph “diamond” probe phase**

This part of the experiment examines two things. It can be considered to probe the formal nature of the hippocampal code for environments, in so much as the data can
test predictive models of hippocampal firing (Hartley et al, 2000) in various environments. The Hartley et al (2000) model requires more than two shapes in order to be predictive. The morph "diamond" adds another shape, and may be considered to test if there is anything particularly salient about corners, as has been suggested by some investigators (eg. Mittelstaedt - personal communication by Neil Burgess). This also tests if the experimental attempts to "anchor" the head direction system have been successful.

The results from the morph diamond probe phase of experiment 1 were clear cut. Figure 8h is representative of the results from all rats tested in experiment 1A and 1B. A clear majority of the cells fired in positions that were predictable on the basis of their positional firing in the morph circle and morph square.

It is of particular importance to note that the angular location of the place fields in the diamond is not predicted by simply rotating their position in the morph square by 45 degrees in any direction. This result is obvious in cells 1, 3, 5, 6, 11, and 12. Cell 7 may seem like an exception, but the peak in the north-east corner of the square may well have been associated with lower sampling of the north-east corner, and moreover, the position of the field in the diamond is well predicted by its position in the circle. The results, even though predicted by the experimenter, are striking. The results suggest an almost ideal anchoring of head direction sense by the experimental procedures. Accordingly, when shape-based remapping is seen (eg. in Figure 8g), one may be reasonably confident that this remapping is not subsidiary to altered firing in head-direction system inputs to hippocampus. As such, these trials represent an important control in the process of establishing specifically hippocampal learning.
Experiment 1A and 1B - Probe trials examining the basis of the similarity of firing

Introduction

This is a large topic, and cannot be fully explored in this section, owing to considerations of space and the focus of this thesis upon learning. An abbreviated treatment of the results is given here. One important overall result, however, must be taken from this section. It is that the similarity of across-shape firing seen in the main experiments, where it is seen, is not simply due to the fact that the animals are in the same laboratory testing space. Two kinds of arguments could be used to assert this view. These may be represented as follows:

1) “I don’t care that you changed the floor paper, the fields represent smells coming through the paper, it’s trivial that the fields are in the same place. Also you use the same morph box. And even if it’s not the smells, well the cells are laboratory arena cells, responding to other “real world” aspects, like views of the cue card, and overhead camera which change depending on where you are in the environment.”

2) “You always place the animals in the centre of the environment, after a similar passive displacement from the holding platform; the fields reflect path integration processes”.

Probe trials - types

Various trials were performed to test, inter alia, if room cues, path integration, and so on could account for the results. This section will show that neither of the above two objections is tenable. Figure 8II shows a subset of the trials performed on r977, which draws attention to the variety of configurations of the morph box which r977 was
Configurations of the morph box

TRAINING (3 DAYS)

10 trials in shape a

DAYS 1 TO 6

6 consecutive days
6 trials per day
(3 in a, 3 in b, alternating)

PROBE PHASE
DAYS 7 TO 18

The probe phase included the first trials in enclosures c to h
Recording was not always on consecutive days

g barrier = 9cm thick
h barrier = 4.5 cm thick
both = 28cm high

Figure 8il. Diagram of sequence of testing, and the various shapes and configurations tested, for r977. Several kinds of trials (eg. translation, and directional sense, probe trials) are not represented here.
Place cell firing in several different morph box configurations

Figure 8ii. Firing of two cells in 8 different configurations (labelled a to h) of the morph box.

Firing rate maps are shown for two place cells recorded over an 18 day period, from rat r977. There are essentially six different shapes (a to f). Two other configurations (g and h) were created by placing central barriers in the large square and small square.

An asterisk denotes the trial in which the rat was first exposed to the particular shape shown.

Note that both cells have ostensibly similar fields in the small morph square (shape a) but the probe trials in different shapes seem to bring out differences in their “underlying” inputs. Most notably, cell 2 fires neither in the NE triangle (shape f) nor above the barrier in configurations g and h.
tested with. Translation trials, and directional probe trials were also performed on this and other animals.

Results

There are many results which undermine both interpretations caricatured above, and not all the data will be described or shown:

a) When two morph boxes are used to create a larger morph square, fields that are close to morph walls stay close to walls (eg. see Figure 8iII, shapes a and d). This result is similar to a translation which results in similar intra-morphbox patterns. This is clearly inconsistent with room and local cue interpretations of similar firing. It is inconsistent with the above path integration account of similar firing, since the centre of the larger morph-environment is still the same. If, for example, a field was responding to being about 30 cm due south from the centre, why is it not still there? As Figure 8iII shows, the fields stay with the wall.

b) Translation trials of 20 cm and/or 40 cm of the morph square were performed, and the results showed similar intra-morphbox patterns (data not shown).

c) When various shapes inhabiting parts of the same room space are created, the firing patterns are not "more or less" predicted by room space, when the morph configurations are quite different. See Figure 8i, and examine firing across the set of morph configurations a, d, f, g, and h for cell 1. To pick one egregious example, configuration f forms a part of the space occupied by configuration d, yet the fields in these two configurations are in completely different places in room space.

d) It is possible (though difficult because the rat's direction sense has been well anchored) to change nothing in arena space, and to alter the angular location of fields within the intramural space. This was done for one animal, whose rate maps are
shown in the previous two figures (r977); it was possible to rotate the fields. This involved a long series of trials, but the following summary is all that is required here.

1) Very slow (presumptively less than vestibular threshold) angular rotation of the rat, 2) in darkness, and 3) entering the rat into the square over the east side of the square not the south, in combination, (neither one nor two of these conditions alone sufficed) produced a 90 degree rotation of the fields.

e) Finally, again for one animal only in this experiment (r1039 from Experiment 1A), replacing the paper floor with a Trespa platform, and raising this platform off from the floor by 27cm (i.e., a y-axis translation) produced similar patterns of firing as in the standard set up. (Interestingly, the animal did rear more.)

In all, the results strongly indicate that the homotopic firing patterns in the main experiment relate to the similarity of the morph square and morph circle, and are not a simple function of the similarity in arena space. This is not to deny that the same morph box in a different part of the lab space can produce remapping (as was indeed seen in the pilot experiments previously described).

**Experiment 1B - Delay testing**

One animal from Experiment 1B, r1062, was tested after a 32 day absence from the curtained environment. Animal r1062 underwent the normal 6-day time series and some further probe trials. Figure 8j shows firing rate maps from the 5 cells recorded from this animal after this delay. The fields are clearly similar across shapes. Such a result, though only from one animal, strongly suggests that time itself, without actual
experience in the environments, does not induce remapping beyond that already seen
in the main experiment, at least not in the type of paradigm used here.
**Time itself does not induce remapping**

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<td>2.6</td>
<td>2.4</td>
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Fig 8j. Firing rate maps from animal r1062 after a delay period show no evidence of remapping.

32 days had elapsed since this rat had been exposed to the curtained environment. On the day previous to this delay test, the rat had been brought into the laboratory room for an hour and a half. If anything, such a procedure should help retrieve expectations of intra-curtain environments, and thus aid any potential remapping.

This delay trial suggests that a long passage of time without actual experience in the environments, is not sufficient to bring about remapping.

Trials shown are first two trials.
Thus when cells do start to show divergent patterns of firing across shapes, it is highly probable that they do so primarily because of environmental experience. If there is a role for the discriminatory manipulation of remembered experience when the rat is outside the environments being remembered, perhaps it can apply only limited amplification of what intra-environmental discriminatory tendencies are implicit in the stored patterns; certainly in this paradigm at any rate, where there is no reward for producing remapping-like firing patterns. However, the simple conclusion we wish to draw from this result is for the purposes of experiment 2; it is unlikely that some mysterious entropic force can cause a divergence in across-shape patterns through a long passage in time. So, where we see remapping occur, and patterns remain remapped after a long delay, this may be considered to reflect memory storage processes, and not some variant of Murphy’s law as applied to homotopy. This is an important conclusion to take to the results of experiment 2.

**Experiment 1 - discussion**

For brevity, this discussion focuses on the main result. The pilot experiments had strongly suggested a view of remapping which was formally tested in experiment 1. This view can be expressed as follows: **During initial exposures to one walled environment similar in all respects save shape to another walled environment, the firing patterns of hippocampal place cells in both the environments will be similar.**

This view was confirmed by the results. Indeed, although the analysis was not performed animal-by-animal, firing rate maps were presented from all the cells
recorded from all the seven animals, and no animal appeared exceptional. The results were clear and universal. Furthermore, it was shown that the similarity of firing patterns did relate to the walls of the boxes.

Any generalisations based on this view need to attend to the following qualifications:

1) The phrase “similar in all respects” does include the idea that the shapes are in the same position in the lab, and that no attempt is made to disabuse the rats of such a notion. Note, as stated above, that similarity of room position cannot, alone, account for the similarity in firing patterns across shapes.

2) The shapes in question do not in any way encourage the rats’ direction system to alter its firing patterns (as might occur with, say, a rectangle, and an equilateral triangle with no sides parallel to the rectangular walls.) As described in Chapter 4, head direction cells can alter their firing patterns in different shapes in the same kind of testing conditions used for place cell recording.

Other hypotheses only hinted at by the results of the Pilot study concerned repeated exposures. Specifically, where shape remapping occurred: 1) an important trigger might be repeated experience of both environments; 2) the remapping would not occur in an all-or-none one step fashion. These ideas were looked at in experiment 1B. Qualitative evidence in support of these hypotheses was produced. Because none of the three animals taken to day 6 in Experiment 1B showed a majority of remapped cells on day 6, a new experiment with new animals was designed, in which the limit of repeated exposures was greatly increased. This was experiment 2 (see next chapter, Chapter 9).
CHAPTER 9:

EXPERIMENT 2

Experiment 2 - Rationale and aims

Building on the results of experiment 1, experiment 2 was specifically designed to examine the effect of experience in repeated exposures going up to as far as 21 days, if necessary, to see if more complete remapping would occur. Furthermore, the experiment would now examine cell responses right from the very first exposures in the environments, with no training period as such, such that place cells would be recorded for the entire duration of the rats’ experience in the environments.

The hypothesis was explicitly tested that the similarity of firing-across-shapes would decline incrementally in an experience-dependent fashion. It is fair to say that the experimenter was far from expecting complete remapping (thus the 21 day limit), only that where remapping did occur it would do so as stated above.

It is useful to define the term “incremental” as used in the present thesis.

“Incremental” is used here to point out that in the paradigms studied in this thesis, the remapping process is not like that seen in other studies such as in Bostock et al (1991). These authors investigated the transition from similar to dissimilar firing, reporting “our results indicate that the transition is abrupt; we saw little evidence of a gradual shift through partial remapping” (Bostock et al, 1991). In practice, how fast was abrupt? Bostock et al showed evidence suggesting a transition from similar to remapped patterns in about 3 minutes. (There are sampling problems associated with demonstrating that it is much quicker than this.) This is with regard to individual cells.
Where pairs of cells were recorded, the firing patterns were either similar or remapped. Further, the results showed that once one cell showed remapping patterns, all subsequently recorded cells showed remapping, implying a complete all-or-none remapping transition for the population of cells. The present thesis shows different results. First, regarding the population of cells recorded, remapping is incremental in the sense that the averaged index of remapping over the whole recorded sample increases. In principle, this could occur with all the individual cells making abrupt remapping transitions. However, evidence is presented showing that some individual cells can take much longer than 3 minutes to make a transition from similar to dissimilar firing. The first claim then is that the population remaps incrementally, and the second is that some cells remap incrementally over a time period of days. This has important implications for the study of learning at the cell and synaptic level.

This is not to deny that the transition for a subset of the cells may be abrupt. Moreover, it would be expected that at least some cells show some stability after a time. For instance, regarding field peak position, the field peaks of a cell that fires in the south-west of the square and the north-east of the circle cannot get any further apart from each other. At any rate, these issues have not been fully investigated. However, it may simply be said that the definition of incremental is such that if, for example, a cell shows, on a dimension of similar/dissimilar firing, relative constancy for a period of 3 days, gradual decrease for 3 days, and then constancy for three days, this may be called incremental.
There were several other aims in experiment 2, but perhaps only three major aims need be mentioned here, all relating to the anticipation of more “thorough” remapping at the end of the main time-series experiment. Three questions were posed.

1) Would the incidental learning of the two environments associated with more thorough remapping “cross over” in any way to the representation of other similar environments (specifically those of the same size and shape, but constructed from the morph box of experiment 1.) Was there evidence of Tolmanian knowledge-transfer?

2) Would the memory of incidentally learned information associated with more thorough remapping be stable, as suggested by retesting animals after a delay period of about a month?

3) What, exactly, would be incidentally learned, and how might the process occur? Is remapping always “arbitrary” (as most models assume), and if not, is it ever predictable?

**Methods - Experiment 2**

The subjects in experiment 2 were r1079, r1029, and r1077.

**Experiment 2 – Environmental set up**

The setup used in experiment 2 is shown in Figure 9. It can be seen it was very similar to experiment 1, except that:

1) the shaped environments were wooden and painted
2) the shaped environments were placed on a raised platform.
Experiment 2 - Setup and sequence

A) Setup

The setup is similar to that of experiment 1, but an internal cue card is used, on the laboratory north side of the circle or square box, and the current box is placed on a platform raised from the floor.

B) Testing sequence

Day 1 and 2

Circle 1  Square 1  Circle 2  Square 2

Day 3 up to day 21

Circle  Square  Circle  Square  Circle  Square

Figure 9. Diagram of laboratory setup and testing sequence used in experiment 2.

A) The setup
The setup is similar to that of experiment 1, but an internal cue card is used, on the laboratory north side of the circle or square box, and the current box is placed on a platform raised from the floor.

B) The testing sequence
Circles and squares alternate. There are 4 trials a day on days 1 and 2, and 6 trials a day on all other days.
Four environments were used. These were two square walled, and two circular walled, light grey-blue painted wooden boxes (hereafter referred to as the square boxes and the circle boxes.). The square boxes were 59 cm x 59 cm x 50 cm high. The circle boxes were 77/78 cm in diameter and 50 cm high. These sizes were meant to correspond to the sizes of the morph square (62 cm sides) and morph circle (76 cm diameter). Thus, as it turned out, the square boxes were slightly smaller than the morph square.

Four white cue cards were in use on any one day. Each cue card was 50 cm high and 58 cm wide. One card was stuck (with double-sided sellotape) onto the internal wall, on the lab north-facing side, of each square and each circle. These cards were replaced by a fresh set of four cards after 12-13 days, and if any card had become particularly distinctive through dirt or folding, it was replaced with a freshly made card. The cards were interchanged among the environments, so that each card was used in each of the four environments equally or roughly equally. Two cards each day were used twice.

The final difference from experiment 1 was the use of a black Trespa platform 90 cm in diameter raised 27 cm from the floor. Trespa is a complex synthetic material which is not easily scuffed, and which is easily washed. (The platform was raised from the floor so that probe trials could be done where the walls were removed and the animal would have to traverse the platform.) *The platform was washed between every trial.*
**Experiment 2 – Procedural details**

The testing procedure was very similar to that of experiment 1b. The main experiment consisted of 6 alternating trials of the circle and square boxes, always beginning on every day with one of the circle boxes, for up to 21 days. The experiment was run entirely on consecutive days. This was for two reasons. Firstly, to help follow the same cells over time. Secondly, I felt that any gaps such as at the weekend would cause an extra layer of interpretative difficulty. For instance, if there was an increase in homotopy from Friday to Monday, might this be due to "forgetting"? At any rate, it was felt that simplicity and standardisation were extremely important in this experiment.

There was no "training" phase in experiment 2, in the sense of a phase separable from a testing phase. All trials were analysed. However, because rapid and even traversal of the environment takes time to develop in rats, with familiarity and exercise, rats were run for only 4 trials on days 1 and 2. In other words, it was considered that it might be difficult to get the rats to run 6 trials on the first two days. From day 3 up to day 21, rats were run for 6 trials.

The order of boxes was alternated across days. Thus on day 3, the order was 1st circle, 1st square, 2nd circle, 2nd square, 1st circle, 1st square. Then on day 4 it was 2nd circle, 2nd square, 1st circle, 1st square, 2nd circle, 2nd square. The order on day 5 was like the order on day 3 and so on.

The time-series experiment stopped when complete remapping occurred or 21 days had elapsed, whichever occurred first. The time-series experiment was terminated on
day 6 for r1029, when complete remapping had taken place. The other animals were taken to 21 days. (The cell yield from r1077 was rather low on days 6, 7, and 8. The tetrodes were moved on the night of day 8, to record from more cells on subsequent days. In order to attain stability, no recording was done on day 9. (Some cells were lost, others gained, with a net gain, as desired.) The analysis has ignored this day9-gap. Thus, for r1077, “day 9” was in fact the 10th day, and so on.)

As an additional control over olfactory cues beyond washing the Trespa platform between every trial, the Trespa raised platform was rotated between trials on certain days. On days 5 and 6, the platform was rotated a multiple of 90 degrees between every trial, and on days 7 to 10, the platform was rotated by a multiple of 90 degrees between every two trials. Further, the starting orientation of platform was generally different between days. No effects of these rotations were seen, implying that the cleaning of the platform between trials was effective, or that olfaction was not important.

For ease of reference, the 6th day for r1029, and the 21st day for the other rats in experiment 2 is sometimes called DayEnd.

**Experiment 2 – Morph Transfer Phase – probing the nature of remapping**

On the recording day immediately after DayEnd (day 22 for r1079, day 23 for r1077, day 7 for r1029), rats were exposed to the morph boxes. The procedures differed slightly, but always involved four baseline trials in the two circle and two square boxes sandwiching trials in the morph circle and morph square. Sometimes, in these trials, an extra minute or so was added to the standard 8 minutes in square, and 10
minutes in circle. In every case, the whole trial was used to derive the place field characteristics.

**Experiment 2 - Observations of rearing**

No trials were videotaped. However, it was observed and noted down that increased rearing, and other indices of presumptive exploration, occurred in certain situations in several types of trial involving significant environmental change. For two rats during the Morph Transfer Phase on DayEnd+1, the number of rearing events per trial was counted. In addition, rearings were counted for r1079 throughout the duration of the entire experiment. (Obviously, mere number counts cannot take into account the duration of each rearing event. An observed trend was that the average length of a rearing episode also increased somewhat with environmental change.)

**Experiment 2 – Delay phase – testing long-term memory**

An important component of experiment 2 was retesting the subjects in the original setup used in the main experiment after a substantial delay (given below) *during which the subjects were given no experience whatsoever in the curtained environment*. This delay began after probe trials further to the Morph Transfer phase. Animals were given two trials in each shape, thus four trials in all. The delay for each animal was as follows.

R1029: 28 days since last exposure to laboratory (kept in home cage throughout).

R1077: 39 days since last exposure to laboratory (kept in home cage throughout).

R1079: First delay test 17 days since last exposure to curtained environment, 14 days since last exposure to laboratory (kept in home cage throughout). 2\textsuperscript{nd} delay test 29 days since last exposure to laboratory (kept in home cage throughout).
Results of the Monte Carlo simulation for Experiment 2

The second Monte-Carlo simulation, based on a 200x200 grid, gives a 5% probability cutoff distance of 25 pixels. Accordingly, where applicable to the analysis of combined data, a homotopic cell as defined by the CPCP measure and the Monte Carlo simulation was one where the CPCP distance was less than or equal to 25 pixels.

Results Overview, and Plan of Results

There are quite a lot of results in experiment 2, and it may be useful to give a very broad outline of the whole experiment before immediately launching into details. The results section is divided into six main parts, following the actual order of the experiment, except that probe trials are presented at the end of the chapter. The actual order of the experiment was as follows:

a) The pure Time-series experiment, with animals in the two different shapes from day 1 to DayEnd (DayEnd is on day 6 for r1029, and on day 21 for 1077, and 1079). The basic result is that the patterns are initially similar, but diverge with repeated experience in the shapes.

b) On the next recording day after DayEnd, the animals were placed in the morph circle and morph square, for the first time ever. This part of the experiment is called the Morph Transfer phase. The basic result is that the patterns in each shape can show both similarities and differences from those in the respectively-same-shaped wooden enclosures, but it is always the case that the patterns in the morph square and morph circle are different from each other. This is contrasted with the results of the initial exposures to the identical morph square and morph circle in experiments 1A, and 1B,
where on initial exposures to morph square and morph circle, the patterns were clearly very similar, as has been shown in the previous chapter.

c) After the Morph Transfer Phase, various probe trials were conducted, not all animals receiving the same types of probe trial.

d) After probe trials, animals were kept away from the curtained environment (and thus of course any experience of the two shaped environments) for a substantial delay period (mean = 28 days). This is called the Delay phase of the experiment. After the delay, the animals were placed back in the usual wooden environments, and run as normal, for two trials in each shape. The basic result is that the across shape patterns are still markedly different, and not distinguishable from those on DayEnd.

e) Further probe trials were run after the Delay phase, for some animals.

For the purposes of description, any probe trials in c) and e) are discussed at the end of the chapter. The results part of the chapter, then, is organised as follows:

1) **Day 1 to DayEnd time series experiment - general.** Results are presented qualitatively for animals and for single cells. A general idea of the results emerges.

2) **Day 1 to Day 6 - time series experiment - combined data.** This part combines the data from all three animals up to the last legitimate date for doing so, day 6, the DayEnd point for r1029. Quantitative results are presented.

3) **Day 1 to DayEnd time series experiment - individual rats.** This part examines the time series data individually for each rat.

4) **Morph Transfer phase.** This part is not quantitative.

5) **Delay Phase.** Here the results for Day 1, DayEnd, and the Delay Day, are all compared.
6) **Probe trials.** This part is not quantitative, and tries to account for some of the results seen.

**Experiment 2 - Results**

1) **Day 1 to DayEnd time series experiment - General, qualitative results**

Firing rate maps were created for all the cells in all the animals in all the trials, and from these, the data of field peak positions and field peak rates were entered into spreadsheets. The spreadsheet field peak position and rate data is the basis for all the quantitation that follows later in this chapter. Inspection of the firing rate maps strongly suggests that firing patterns were initially similar across the two shapes but diverged with repeated exposure to the two environments. In other words, the animals did not remap on initial exposures but did so on later exposures.

Figure 9a shows the firing rate maps for all 8 cells recorded from r1079 on day 1. Clearly the across-shape patterns are rather similar. In contrast, the firing patterns across shapes seen in Figure 9b, showing firing maps from day 18 from the same animal, are rather dissimilar. Day 18 was chosen as a representative example for a figure of “remapping” before quantitative analysis was performed, partly because many cells were recorded (n = 19). Without anticipating the analysis too much, the figure may be said to be fairly representative of its point in the time series, both qualitatively and quantitatively.

Figure 9b usefully illustrates examples of all kinds of across-shape pattern. We may consider three ideal categories, though of course this is to impose discreteness upon a continuum. There are cells which fire in one shape but not the other (monotopic: eg. 
No remapping on Day 1

Figure 9a. Firing rate maps from all the 8 cells recorded from r1079 on day 1 in Experiment 2.

The firing rate maps are taken from the middle trials of day 1. For the last cell (bottom row), the first trial is also shown on the extreme left. The general across-shape pattern is not indicative of remapping.
All cells recorded from r1079 on day 18:
Most cells have remapped

Figure 9b. Firing rate maps from all the 20 cells recorded from r1079 on day 18.

The figure has been organised so that cells firing in one shape only over the whole day are shown on the top rows. Cells firing at least once in both shapes are shown on the bottom rows.
Cells 18, 19, and 20 did not fire, in the square trial depicted, above threshold. However, they did fire on one or more of the other square trials, and one firing rate map taken from one of these other trials is shown top right of the appropriate square.
cells 1 and 7), cells which appear to fire in similar positions (homotopic: eg cells 15, and 17), and cells which fire in different positions (heterotopic: eg cells 14 and 18 (inset)). And there are cells which one hesitates to place in any one of these categories firmly. (The fact that remapping in the two shapes takes on two forms, monotopy and heterotopy, while similar firing has only one basic form, homotopy, does complicate the analysis of remapping.) Most cells appear to have monotopic patterns. In this animal, monotopy, and remapping by rate differences across shapes in general appeared to be the dominant pattern in the remapping process.

Is there a remapping "process" that can be observed? After seeing Figures 9a and 9b, the obvious question arises: what happened in between day 1 and day 18? It would be tedious to show all the firing rate maps for this animal from day 1 to 21. Instead, the overt aspects of the remapping process may be seen in r1029, whose DayEnd was day 6, with accordingly fewer firing rate maps to show (See Figure 9ci).

Before commenting on further aspects of Figure 9ci, it should be noted that, for this animal only, the combination of the stability of the recording preparation, and a relatively quick remapping process, meant that the experimenter could follow (or claim to follow) each cell over the recording period, and, for instance, three cells from day 1 to DayEnd and beyond. These three cells are the cells represented in the three left-hand columns. Figure 9cii shows the tetrode spike scatter plots on successive days for the tetrode from which most of the cells were recorded (all cells except those represented in the box titled "Tetrode B"). All the spikes of a certain cell are represented by dots of a given colour, with the colour constant over days. The grey dots represent uncut spikes, ie. more specifically: a) noise; b) any remaining uncut
Figure 9ci. (See opposite page.)

Figure 9ci, on the facing page, shows firing rate maps from 10 cells recorded from days 1 to 7 from animal r1029. The trials from day 7 are taken from the "morph transfer phase" of Experiment 2.

Each day is represented by two trials from that day. Thus for every day, there is a pair of rows of cells in the circle and square.

Each cell is represented by a column going down the page.

Inset shows two cells recorded on day 5, and one on day 6, from another tetrode.

A firing rate map with a dotted line circumscribing it indicates that this was the only trial in which the cell fired.

Above and right of some firing rate maps are shown smaller firing rate maps. These are to indicate that though the cell did not fire at or above the 1.0 Hz threshold in the trial shown, the given cell did do so on another trial, that which is shown above and right.

Optional details
This figure was prepared before the topological transformation of the circle data into square data had been developed. Some cells shown firing at or above 1.0 Hz in the circle in this figure were subsequently rejected from the analysis because the circle-transformed-into-a-square was used for quantitative purposes, not the circle, and some cells did not fire at or above the 1.0 Hz threshold in the transformed-circle on two or more trials. The topological transformation tends to reduce the field peak rate (see Methods.)

The trials shown were all temporally adjacent, except for day 7, where this is not possible.
Position of spike clusters in the space of the tetrode scatterplots is similar - a basis for claiming to follow the cells shown in Figure 9ci over time.

Figure 9cii. This figure presents evidence that the cells shown in columns in Figure 9ci are the same cells.

Every spike is represented as a dot on each of the six scatter plots shown for each day. The axis of the plots is the amplitude of a spike on one channel. Thus each scatter plot plots the amplitude of a spike on one channel versus the amplitude on another channel. Because four channels are recorded, there are six scatter plots per day. (See Methods.).

Each cell is represented by a cluster of dots of a specific colour, which is constant over days 1 to 7.

All that needs to be appreciated is that dots of a certain colour remain in roughly the same position in the tetrode scatter plot space. This occurs in situations of relatively good recording stability.
spikes from the cells shown; c) theta cell spikes; and finally d) uncut spikes from cells
which did not produce place field peaks with rates above 1.0 Hz on two or more trials
on one day at some stage in the experiment from day 1 to day 6 and were thus
ignored). Figure 9cii shows that dots of a particular colour occupy roughly the same
locations in the tetrode scatterplot space from one day to the next. Waveforms have
not been shown but tell the same story.

Thus we may return to Figure 9ci. Firstly, speaking broadly, two features are obvious:
the remapping patterns are dominated by monotopy (though there is one clear
heterotopic cell in tetrode B on days 5 and 6); secondly, it is very clear that the
sampled population does not remap in an all-or-none fashion, but incrementally.
We can compare rates of remapping for individual cells, observing that there is no
unique day when all the across-shape patterns “uncouple”. The cell in the left hand
column (cell 1) does not become “silent” (ie. below the 1.0 Hz threshold) in the circle
until day 5. The cell in the next column (cell 2) is silent in the square from day 2, and
remains so until the end of the experiment. The cell in the fifth column (Cell 5), first
encountered on day 2, becomes silent in the square until day 4, remaining so until the
end of the experiment (note that it does not fire above threshold in either the circle or
square on day 5). To repeat, there is no privileged moment when remapping occurs in
this experiment, as judged from firing rate maps. Even if one assigns only two
categories to a cell, remapped or not-remapped, the process never involves a single
time period of simultaneous change. The number of cells showing across-shape
similarity as a proportion of the total cells, declines in increments. Moreover, there
does appear to be a trend whereby the shape in which the field peak is lower on days
when a cell fires in both shapes, predicts the shape in which the cell will become
Cell initially firing in both shapes becomes silent in one shape

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<td></td>
</tr>
</tbody>
</table>

Figure 9d. Example of a cell which fires in both shapes initially, but later fires in one environment only (the circle). A "homotopic" cell becomes "monotopic".
silent in. (This trend was seen in very broad terms for the data as a whole, but has not been analysed quantitatively.)

It also appears to be the case that remapping is incremental for individual cells. This may not be completely obvious from Figure 9ci, and the reader should now turn to Figure 9d, where the data from cell 1 of figure 9ci is shown from days 1 to 5. All trials are shown. The cell fires in the same place in the square on all 13 trials across 5 days. In the circle, it fires in the same place on 2 out of 2, 1 out of 2, 3 out of 3, 1 out of 3, and 0/3 trials, across the same 5 days. If one considers using more of the available information, ie. all the peak rates from all the trials, then we may note that almost any measure comparing circle: square firing shows incremental change. As one example, the ratio of square: circle firing increases incrementally. This cell illustrates well the trend stated above where, to express it oppositely, the shape in which a cell fires most is the shape it will continue to fire in. As such, it suggests that shape-remapping may, in part at least, be predictable. (See Appendix, cell a, where the remapping of this cell over 6 days is depicted graphically, in terms of the amount of total firing in the square, as a proportion of total firing in the square and circle.)

Figure 9ei depicts a cell which initially fires in both shapes and then becomes silent in the square. This cell was followed by the experimenter from day 9 to DayEnd of the experiment (from r1077). (See also Appendix, cell c). Figure 9eii shows the position of the spikes from the same cell in the tetrode scatterplot space. Each scatter plot, and waveform picture, is taken from the middle circle trial of each day.
Figure 9ei. This figure shows all the firing rate maps from all the trials in the circle and square from day 9 to day 21 for one place cell.

For each day, the daily Rate W/A ratio value is given. The shape in which the cell fires more is indicated, C+ meaning the cell fires more in the circle, Sq+ meaning it fires more in the square.

Firing rate maps for peaks of less than 1.0Hz are also shown (in reduced size, to right of appropriate map).
Figure 9ei (continued).
Figure 9eii. Tetrode scatterplot position, and waveforms, of the cell depicted in Figure 9ei.

Each scatter plot, and waveform picture, is taken from the middle circle trial for each day. All spikes are shown in the waveform pictures. For each day, waveforms are shown, from top to bottom, on the 1st, 2nd, 3rd, and 4th channel respectively. The single waveform on the right of all the waveforms is the resultant average shape. It can be seen that the cell occupies a very similar position in the tetrode scatterplot space, and is well isolated.
We return to Figure 9ei. For each day, firing rate maps from all trials are shown. The first column shows the firing rate maps from the circle trial. The second column shows the circle-transformed-into-a-square firing rate maps. The third column shows the square firing rate maps. For further information, fields below 1.0 Hz are shown in reduced size to the right of the appropriate firing field map.

For each day, Figure 9ei shows: the daily Rate difference within/across shapes ratio, as used in experiment 1. For extra clarification, the shape in which the cell fired most over the whole day is designated as “C+” or “Sq+” (circle, and square, respectively).

Before coming to the main points, some incidental observations are appropriate. First, the rates are rather variable. This variability of firing is not uncommon, and is in fact probably representative of the data in experiment 2 as a whole. It may well be the case that repeated exposures in two shapes induces more firing rate variability than exposure to one shape. As for the general rate decrease in this cell, this cell is neither representative, nor unrepresentative. As far as I have been able to determine, no generalisations can be made about total daily firing rates for individual cells, such as that they decrease over time, remain roughly constant, or increase over time. The only reasonable generalisation is that rates are surprisingly variable, given what seems like a rather standardised protocol and rather homogenous behaviour.

The main point of course is that the cell does not remap suddenly, but in increments, and once the cell is silent in the square, it remains so. If one were to judge the remapping in terms of a simple total firing circle/square ratio, days 14, 15, and 16
look like important days in the remapping transition. Note that at least on the first trial in the square on day 16, the cell still seems to be firing in the usual place (see small square to right of larger square). As a bridge towards the quantitative parts of this chapter, the Rate w/a ratio measure is examined here. The Rate w/a ratio shows relatively high values at first (0.99, 0.44, 0.84 in the first three days) and relatively low values later (0.39, 0.25, 0.18 in the last three days). Some of the noisiness in the transition of the rate w/a ratio from around 1.0 and just below to around 0.2-0.3 can readily be explained. Even where the summed circle:square ratio may be quite high, the w/a ratio is sensitive to trial-to-trial variation in the circle. Thus on day 18, because the firing in the circle (transformed) drops to 0.4 on the last trial, which is the same as the median peak rate in the square, the ratio climbs to 0.80. On day 15, because the variation in peak firing in the circle (transformed) is quite low, the w/a ratio drops dramatically to 0.10. (See Appendix – cell c).

All this suggests that we may expect to see some fluctuations in plots of any given ratio against time, and obviously much of the variance in the time-series cannot simply be explained by the factor of experience. In the quantification that follows, no binning over one or more days has been applied to the data.

So far, the examples have focused on cells which diverge in terms of peak rates. Cells firing initially in both shapes have been shown to become silent in one of the shapes: we have seen the evolution of monotopic firing. Figure 9fi shows an example of a cell (from r1077) where heterotopic firing develops. Figure 9fi depicts one trial in each shape, from day 16 to day 21 and day 23 (baseline trials from the Morph Transfer Phase). The trials selected are those with the highest firing rates in that shape on that
Field peaks move apart

Day 16
Day DBP W/A Ratio: 0.69
CPCP distance: 16 pixels

Day 17
0.22
34 pixels

Day 18
0.57
39 pixels

Day 19
0.28
46 pixels

Day 20
0.35
37 pixels

Day 21
0.39
32 pixels

Day 23*
0.55
49 pixels

Figure 9fi. Cell which is initially homotopic becomes heterotopic.

One trial is shown per day in each shape. The trial with the highest peak rate is chosen.
Figure 9fi. This figure shows all the firing rate maps from all the trials in the circle and square from day 16 to day 23 for the cell shown in Figure 9fi.

Day 23*: No recording was done on day 22.
day, the simple idea being to select those trials where more information is available
(the reader may consult Figure 9fii, which depicts firing rate maps from the trials, but
at reduced size). The key thing to note is the way in which the field in the square
appears to move southwards, over days, so that it is finally in the centre of the square.
More subtle, and also with more variability, is the overall tendency of the field in the
circle to move eastwards (though day 21 is a clear exception.) This last point need not
be conceded, it is the movement of the field in the square that is obvious, and
obviously incremental.

Again as a bridge to the quantitative sections, both the daily DBP w/a ratio and the
CPCP distances are given, for each day, in Figures 9fi and 9fii. Again, the numbers
show more fluctuation than visual inspection of the firing rate maps might suggest.
Day 23 does not contribute data to the time-series analysis, but the comparatively high
DBP w/a ratio of 0.55 on this day is once more explained by intra-shape variation (see
its field peaks in the circle on day 23 in Figure 9fii). Anthropomorphising, one can
say that the DBP w/a ratio “punishes” the cell’s field peak variability in the circle (the
overall field is actually rather similar), despite the fact that both field peaks in the
circle are still far from the peaks in the square. It will probably be agreed that the
quantitative measures cannot capture all the subtleties of the transformation as
witnessed by human inspection of firing rate maps, and can obscure the incremental
nature of the remapping.

In the quantitative portions of the chapter which follow, it will be seen that despite
this non-trivial amount of variability in rates and field peak positions, at the level of
individual cells, among different cells, within days and across days, nevertheless very
significant proportions of the variance can be explained by the simple factor of time in
the boxes, i.e. experience.

In summary, examples of firing rate maps over several days have shown that
remapping clearly seems to be an incremental process. An important caveat must be
mentioned. These figures may be misleading if the reader assumes that the
incremental remapping is always a visible process for every cell. It is by no means
possible to follow every cell, and not all cells are ideally isolated throughout the entire
duration of that period during which they are recorded. That is to be expected, more
important is the following. *It is possible that some cells begin to fire for the first time
in the curtained environment, generally later in the time-series, in a pattern that is
already dissimilar across shapes. Similarly, it is possible that some cells which begin
firing similarly across shapes, generally earlier in the time-series, stop firing in both
shapes.* Both such patterns will obviously contribute to, and tend to increase the
gradient of, the incremental remapping process. (Again, the distinction must be drawn
between the incremental remapping for the population, and for the individual cell.)

The demonstration of both these possibilities face the same technical problem. It is
extremely difficult with current extracellular recording technology, to provide
convincing evidence that a particular cell within the range of the electrode is not
firing. The best evidence is when such a cell fires a few spikes only, but it is a simple
fact that confidence of identity increases as the number of spikes increase. Finally,
given that one usually wishes to show that a cell which has stopped firing remains
silent, this problem is faced for a series of days, and the difficulties mount non-
linearly. (It is of less value showing that a cell is not firing on only one or two days:
Table 9.1  Table showing number of cells recorded from each animal per day in the time-series part of Experiment 2.

<table>
<thead>
<tr>
<th>DAY</th>
<th>r1079</th>
<th>r1029</th>
<th>r1077</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td></td>
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</tr>
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<td>10</td>
<td>12</td>
<td></td>
<td>19</td>
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<tr>
<td>11</td>
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<td></td>
<td>12</td>
</tr>
<tr>
<td>12</td>
<td>16</td>
<td></td>
<td>9</td>
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<td>13</td>
<td>16</td>
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<td>14</td>
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<td>15</td>
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<td>18</td>
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<td>14</td>
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<td>19</td>
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<td>14</td>
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<td>21</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>21</td>
<td>22</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Sum</td>
<td>264</td>
<td>30</td>
<td>205</td>
</tr>
<tr>
<td>Mean</td>
<td>12.6</td>
<td>5.0</td>
<td>9.8</td>
</tr>
</tbody>
</table>
this is for the reason that cells sometimes appear to “shut off” and then resume firing *as before*. Although not in the context of remapping, O’Keefe (personal communication) also has seen this shut-off-then-resume-firing-as-before phenomenon. Accordingly, therefore, it must be stressed, that one or two days worth of silence on either side of the above-threshold firing, does not constitute strong evidence, *per se*.) For what it is worth, I suspect that both these processes (homotopic cells shutting off, and cells starting to fire in already-monotopic or already heterotopic patterns) occur, but no data is shown to confirm this. (See Appendix.)

**Sections 2 and 3 - time series experiment results - introductory remarks**

Table 9.1 shows the number of cells recorded from each animal per day in the main, time-series, part of the experiment. The cell-day total was 264 for rl079, 30 for rl029, and 205 for rl077. All cells were recorded from the CA1 region. It may be noted, for each animal, the number of cells recorded later in the time-series is generally higher than the number recorded early on.

There are broadly two ways of analysing the data from these cells.

Section 2 analyses the combined data from all three animals up to the last legitimate day for doing so, day 6.

Section 3 examines each animal individually from day 1 to DayEnd (day 6 for rl029, day 21 for rl079 and rl077). Clearly, the number of cells in the sample for a given day can be quite low for individual animals, However, previous work (eg. Bostock et al, 91), including the time-series data from experiment 1B (eg. compare Figures 8g and 8h), suggests that there can be individual differences in remapping behaviour. We can simply ask - does each animal show the general trend?
Table 9.1b  Table showing number of cells recorded from each animal per day up to
day 6 in the time-series part of Experiment 2.

<table>
<thead>
<tr>
<th>DAY</th>
<th>r1079</th>
<th>r1029</th>
<th>r1077</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>3</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>3</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Sum</td>
<td>37</td>
<td>30</td>
<td>35</td>
<td>102</td>
</tr>
<tr>
<td>Mean</td>
<td>6.2</td>
<td>5.0</td>
<td>5.8</td>
<td>17.0</td>
</tr>
</tbody>
</table>
Combined all-rat, all-cell data: remapping over 1st six days

A) Mean all-cell DBP Within/Across ratio over time

Linear correlation of the all-cell mean ratio against day:
\[ r = -0.91, p < 0.01 \]

B) Mean all-cell Rate Within/Across ratio over time

Linear correlation of the all-cell mean ratio against day:
\[ r = -0.90, p < 0.01 \]

Figure 9g. Incremental decline in both of the all-cell mean Within/Across measures over 1st six days.

Each graph depicts the mean w/a ratio averaged over all cells (A= DBP w/a ratio = green; B= Rate w/a ratio = red). Purely for information, error bars depict standard error of the mean. Note that the correlation is simply for the daily mean from the all the cells against day, and takes no account whatsoever of intra-day, intra-animal, inter-animal variance.

Both curves show an apparently linear decline over these first six days of experiment 2. See text for further details.
2) Day 1 to Day 6 - time series experiment - combined data

Table 9.1b shows the number of cells recorded from each animal per day up to day 6. The total number of cells from each animal is roughly equal, and the total number of cells per day is roughly equal, with the exception that there are only 12 cells for day 6. The mean number of cells recorded per day is 17.0.

The data can be analysed in several ways, depending on how much of the variance we are trying to account for. Three types of analysis will be considered.

First, we can add all cells from all animals together, and consider the daily whole-sample mean of the DBF and Rate w/a ratios. These daily whole sample means can then be plotted against day (see Figure 9g). The initial values for both the DBF and Rate w/a ratios are around unity, and are thus similar to those obtained from experiment 1A and 1B. The ratios decline incrementally. The incremental nature of the decline may be appreciated by performing a linear correlation of the daily sample mean of each ratio (ignoring all other variance) against day for both measures (one-tailed): For the DBF ratio, \( r = -0.91, F(1, 4) = 20.54, p<0.01 \). For the Rate ratio, \( r = -0.90, F(1, 4) = 18.07, p<0.01 \). The correlations are obviously high as well as significant. The w/a ratios are well predicted by knowledge of the day of measurement.

Since the number of cells per animal, and number of cells per day, are broadly similar, there are two further ways of presenting the data. One analysis considers the inter-animal variance, and the other considers all possible variance. Figure 9h depict these.
Combined data (per animal, and all cells): Remapping over 1st six days

A) DBP Within/Across ratios

1) Mean per rat

\[ r = -0.46, p<0.05 \]

B) Rate Within/Across ratios

1) Mean per rat

\[ r = -0.72, p<0.0005 \]

Figure 9h. Scatter plots showing decline in measures of similar firing over 1st six days.

A) shows DBP within/across ratios over time
B) shows Rate within/across ratios over time

For both A) and B), the first scatter plot (1) shows the mean daily value of the given ratio from all available sampled cells per rat. Each of the three rats contributes one datapoint per day. The second (2) depicts the daily ratio of every cell sampled on that day. Underneath each day in (2), the number of cells contributing to the data for that day is indicated.
data graphically. On the left, each value (squares) represents the appropriate mean daily w/a ratio for each of the three animals. On the right, each value (asterisks) represents the daily w/a ratio of each cell. Underneath each plot on the right hand side, the number of cells contributing to the scatter plot is written.

The left scatterplots ignore the inter-cell variance within each animal. Each animal contributes one datapoint per day. The incremental decline in the ratios may be appreciated by performing a linear correlation of these daily per-rat means against time (one-tailed tests). For the DBF ratio, $r = -0.46$, $F(1, 16) = 4.32$, $p < 0.05$. For the Rate ratio, $r = -0.72$, $F(1, 16) = 16.84$, $p < 0.0005$.

Finally, Figure 9h (right) plots all the cells' daily ratio values. (These scatter plots may be compared against Figure 9g, since they depict the individual values contributing to the means and error bars shown in Figure 9g.) The linear correlation (one-tailed) for all the cells' ratio values against day can be examined. The results are as follows. For the DBP w/a ratios, $r = -0.35$, $F(1, 62) = 8.84$, $p < 0.0025$. For the Rate w/a ratios, $r = -0.44$, $F(1, 100) = 23.96$, $p < 0.000025$. (It is remarkable that estimated epsilon squared is 19%, that day accounts for virtually a fifth of the full cell rate w/a ratio variance.) Thus, even with all the sample variance included, there is a good correlation between the respective w/a ratios, and time. The hypothesis of similar across-shape firing becoming dissimilar in stages, is also supported by this quantitative analysis.

In summary, the data show incremental decline in both types of within/across ratio in all the analyses.
"Homotopic" firing patterns decline incrementally

Figure 9i. Incremental decline in homotopic firing patterns over 6 days in Experiment 2.

Each graph depicts the percentage of "homotopic" cells per day, under various measures of what constitutes homotopic firing. The calculation is as follows. For each cell that fires in both shapes, the centroid field peak in each shape is calculated. The Euclidean distance between the centroid peak in the transformed circle, and the centroid peak in the square is noted (CPCP distance). The number of cells with CPCP distances of less than or equal to a given pixel distance (eg. 25 pixels) is divided by the number of cells which fire in both shapes.

The crucial cut off distance, derived from the 200x200 pixel grid Monte Carlo model, is 25 pixels. That is, it may be considered that when the centroid-centroid peak distance is < or = 25 pixels, the cell is firing in a similar place in both shapes ("homotopic"). The proportion of homotopic cells under other cut off values are shown in the graphs, producing similar results.
Recall that there is an inequality in the daily data because there were four trials on day 1 and 2, and six on the rest. In retrospect, the experimenter might have conducted only four trials every day, and ignored the fact that the animals are prepared to run more trials on later days. At any rate, as a check for this, for the combined data only, the last two trials were eliminated from the spreadsheets. These new scatterplots, though based on less data, look similar, and are not shown: the 4-trial only correlations were as follows. For the DBP ratios against time, \( r = -0.28 \) [all trial \( r = -0.35 \)], \( F(1, 53) = 4.4, p<0.025 \). For the Rate ratios against time, \( r = -0.40 \) [all trial \( r = -0.44 \)], \( F(1, 92) = 17.2, p<0.0001 \).

We turn to the other measure of peak-position remapping, the CPCP distance. In combination with the Monte Carlo simulation for experiment 2, we can determine, for each day, the number of cells which may be categorised as homotopic. As previously implied by the DBP data, there are days with rather few cells, but the CPCP measure can also use cells with only across-shape data points. Fewer data points are excluded. Again to try and take account of any possible bias by having 4 trials on the first two days, and 6 trials on the others, both sets of data were considered. The total number of cells with CPCP distances in the all-trial dataset is as follows, from day 1 to 6: 17, 17, 7, 12, 13, and 5. The number in the four-trial dataset is: 17, 17, 6, 11, 11, 3. Figure 9i shows the graphs depicting, for each day, the number of homotopic cells as a proportion of the number of cells with CPCP distances (just mentioned). This is done for both the all-available-trial, and four-trial only datasets. Each graph depicts the curves for several cutoff distances (23,
Experiment 2 - Combined data days 1 to 6 - no correlation of DBP and Rate w/a ratios

Figure 9j. Scatter plot showing the lack of correlation, at the level of each cell, of the Rate Difference Within/Across ratio against the Distance-between-peaks (DBP) Within/Across ratio.

The data points for each day are plotted in different colours. Note that not all cells contribute to this scatter plot, some cells lacking both within and across DBP data points. The number of cells of each day that contribute to the scatter plot are noted in the key to the scatter plot. For instance, "13/19" means that 13 out of 19 available cells had DBP ratios.
are used. Both graphs show that the proportion of homotopic cells declines incrementally, from around 80-90% to around 20-40%.

Figure 9j is a scatterplot of the combined data from each day over 6 days, where each cell’s DBP w/a ratio is plotted against its Rate w/a ratio. As with experiment 1, there is no obvious correlation between the ratios (two-tailed, p>0.66). In other words, a small DBP ratio does not predict a small Rate ratio well, and so on.

In summary of section 2 of the results, although the number of cells is not that high for the combined data on certain days (and low on day 6 in particular), various measures show incremental decline in the similarity of across-shape firing patterns, or to express it differently, incremental increase in remapping patterns.

3) Day 1 to DayEnd time series experiment - individual rats

Analysis - introduction and procedures

In this section, each animal will be examined singly, and in turn. The point of the analysis is to see if each individual animal shows incremental remapping. When analysis of remapping is based on combined data from different animals, it is possible that the picture of incremental mapping obtained might obscure the fact that each animal remaps abruptly, but each on different days. Although various features of the evidence from sections 1 and 2, and the fact that there are only three animals, suggest this is unlikely, it is important to investigate if each animal remaps abruptly.

The results in this section will show that this view cannot account for the data. For each animal, this section will present some evidence for incremental remapping.
As Table 9.1 shows, many more cells were recorded later in the time-series than at early stages. This means it is not reasonable to do an analysis like that performed for the combined data in section 2 (Figure 9h, part 2), where the daily w/a ratio of every cell contributes to the analysis. Only the daily all-cell sample mean is considered. For each animal, the following five sets of variables will be presented graphically, with correlation tests:

i) The daily all-cell sample mean DBP w/a ratio against Day.

ii) The daily all-cell sample mean values of the absolute Within, and absolute Across DBP values, against Day.

iii) The daily all-cell sample mean Rate difference w/a ratio against Day.

iv) The daily sample mean values of the absolute Within, and absolute Across rate difference values, against Day.

v) The daily sample mean DBP w/a ratio vs the daily sample mean Rate w/a ratio.

The primary relationships of interest (i and iii: ie. both the w/a ratios against Day) are tested for correlations using both linear and logarithmic fits. The correlations of other variables are investigated for linear relationships only. The experimental predictions relating to two-tailed and one-tailed tests were as follows. Measures of remapping would increase over time. The most important experimental prediction to test is that the w/a ratios decrease over time (one-tailed test). In this section, the averages of the within and across values were also plotted against time, for further information. The predictions here are less important to the thesis. The prediction was that the within measures would be roughly constant (therefore any deviation from this is a two-tailed test), while the across values would increase (one-tailed test).
Table 9.2. Correlation Table, presenting various correlation statistics associated with experience-dependent remapping in the animals of Experiment 2. The important variables to be tested are highlighted in bold. For these variables, both logarithmic and linear correlation models were fitted to the data. Adjusted R squared, or estimated epsilon squared, is abbreviated as “AdjR^2”. “DBP” is an abbreviation for distance-between-peaks. All tests are one-tailed, except the two-tailed tests indicated by two asterisks “**”.

<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>VARIABLES Correlated</th>
<th>LINEAR Model</th>
<th>LOGARITHMIC Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1079</td>
<td>1) Day &amp; 2) W/A ratio for rate diff.</td>
<td>( r = -0.68, F(1, 19) = 16.61, \ p&lt;0.0005, \ AdjR^2 = 0.44 )</td>
<td>( r = -0.74, F(1, 19) = 23.62, \ p&lt;0.0001, \ AdjR^2 = 0.53 )</td>
</tr>
<tr>
<td></td>
<td>1) Day &amp; 2) W/A ratio for DBP</td>
<td>( r = -0.58, F(1, 19) = 9.74, \ p&lt;0.005, \ AdjR^2 = 0.30 )</td>
<td>( r = -0.75, F(1, 19) = 24.37, \ p&lt;0.001, \ AdjR^2 = 0.54 )</td>
</tr>
<tr>
<td></td>
<td>1) W/A ratio for rate diff &amp; 2) W/A ratio for DBP</td>
<td>( r = 0.70, F(1, 19) = 17.79, \ p&lt;0.001**, \ AdjR^2 = 0.46 )</td>
<td></td>
</tr>
<tr>
<td>R1029</td>
<td>1) Day &amp; 2) W/A ratio for rate diff.</td>
<td>( r = -0.78, F(1, 4) = 6.41, \ p&lt;0.05, \ AdjR^2 = 0.52 )</td>
<td>( r = -0.78, F(1, 4) = 6.21, \ p&lt;0.05, \ AdjR^2 = 0.51 )</td>
</tr>
<tr>
<td></td>
<td>1) W/A ratio for rate diff &amp; 2) W/A ratio for DBP</td>
<td>No significant correlation, \ p&gt;0.18 )</td>
<td>No significant correlation, \ p&gt;0.24</td>
</tr>
<tr>
<td>R1077</td>
<td>1) Day &amp; 2) W/A ratio for rate diff.</td>
<td>No significant correlation, \ p&gt;0.22 )</td>
<td>No significant correlation, \ p&gt;0.17</td>
</tr>
<tr>
<td></td>
<td>1) Day &amp; 2) W/A ratio for DBP</td>
<td>( r = -0.50, F(1, 19) = 6.27, \ p&lt;0.025, \ AdjR^2 = 0.21 )</td>
<td>( r = -0.59, F(1, 19) = 10.18, \ p&lt;0.0025, \ AdjR^2 = 0.31 )</td>
</tr>
<tr>
<td></td>
<td>1) W/A ratio for rate diff &amp; 2) W/A ratio for DBP</td>
<td>No significant correlation, \ p&gt;0.69** )</td>
<td></td>
</tr>
</tbody>
</table>
"Remapping" over time: r1079

A) MEAN DISTANCE BETWEEN PEAKS (DBP)

A1) Absolute Mean Values: Within & Across Shape DBP over time

A2) Within Shape DBP/Across Shape DBP Ratio over time

B) MEAN RATE DIFFERENCES

B1) Absolute Mean Values: Within & Across Shape Rate differences over time

B2) Within Shape Rate difference/Across Shape rate difference Ratio over time

C) Rate Within/Across ratio Vs Peak Distance Within/Across ratio

Figure 9k. Graphs and Scatter plot depicting various relationships associated with "remapping" over time in animal r1079 of Experiment 2.
Note that in Experiment 1, the absolute Within and Across values were compared against each other. Here in Experiment 2, the point of interest is to compare these values against Day (ie. experience). Note that with remapping situations there may be several cells feeding into the average absolute within DBP values which do not feed into the average DBP w/a cell ratios because they do not have any across-shape datapoints.

Results

Animals will be considered in the following order: r1079, r1029, r1077. Figure 9k presents the five sets of variable-relationships for r1079, Figure 9l the same for r1029, and Figure 9m for r1077. The correlation table, Table 9.2, summarises the correlation statistics for each animal for the major variables of most concern (i, iii, and v in the list above, ie. omitting the absolute within and across values, and examining the W/A ratios only). F values, and degrees of freedom not shown in the graphs are presented in Table 9.2.

Figures 9k (r1079), 9l (r1029) and 9m (r1077) are all identical in presentation, and thus provide a concise means of comparing the data across the different animals. To avoid clutter, the correlation statistics for the absolute within and across values against day are not shown on these figures. In all figures, Across measures are shown in blue, within measures in brown, DBP w/a ratios in green, and Rate w/a ratios in red. Figure 9k will be explained in detail first, and the general principles will apply to the other two figures.
Animal r1079

Figure 9k presents the data associated with remapping for r1079. Part A) presents DBP-related measures. Part B) presents Rate difference-related measures. Part C) is a scatterplot showing the daily mean DBP w/a ratio value against the daily mean Rate w/a ratio value.

Part A1) of Figure 9k shows the absolute daily mean values for the Within and Across DBP measure. Both measures begin by being roughly equal. The across DBP value (blue line) increases over time, while the within DBP value (brown) remains roughly constant. This is as expected. There is no significant correlation between the within DBP value and time (two-tailed, p>0.54), but there is between the across DBP value and time (one tailed, r = 0.52, F(1, 19) = 7.03, p<0.01).

The mean DBP w/a ratio curve decline (Figure 9k:A2) shows a better fit to a logarithmic than linear function (one-tailed: linear: r = -0.58, p<0.005; logarithmic: r = -0.75, p<0.001, see Table 9.2 for F values etc.)

Part B1) of Figure 9k shows the absolute daily mean values for the Within and Across Rate difference measure. The across rate difference value (blue line) remains roughly constant, while the within rate difference value (brown) shows a significant decline. This was not predicted, though the result is by no means surprising. It might suggest that the cells become more stable in terms of rate with repeated experience. There is no significant correlation between the across rate value and time. The linear fit actually shows a slight decline over time in the across rate differences. The two-tailed probability of the decline is p>0.32. The within rate difference value shows a strong,
significant negative correlation with time (two-tailed, $r = -0.75$, $F(1, 19) = 23.83$, $p<0.00025$).

The mean rate w/a ratio (Figure 9k:B2) shows a somewhat more logarithmic than linear decline over time (one-tailed: linear: $r = -0.68$, $p<0.0005$; logarithmic: $r = -0.74$, $p<0.0001$, see Table 9.2 for $F$ values etc.)

Accordingly, r1079 shows clear evidence of both peak rate and peak position remapping over time. The correlation against day for each ratio for the logarithmic fits is basically the same ($r = -0.75$ and -0.74). This may explain why the daily mean DBP w/a ratio correlates well with the daily mean rate w/a ratio (see part C) of Figure 9k.) Part C shows the scatterplot of the daily ratios, showing that smaller mean daily DBP w/a ratios tend to predict smaller mean daily rate w/a ratios, and so on. There is a strong positive linear correlation between the two ratios (two tailed: $r = 0.70$, $F(1, 19) = 17.80$, $p<0.001$).

**Animal r1029**

Figure 9l presents the data associated with remapping for r1029. Part A1) is somewhat misleading. As this animal’s remapping patterns were dominated by monotopy (there was one heterotopic cell on day 5 and day 6 - see Figure 9ci again), and not that many cells were recorded from this animal, very few cells contribute to the across measure (blue line). The number was as follows, from day 1 to 6: $n = 3, 1, 2, 1, 2,$ and 1 respectively. The jump from day 4 to day 5 reflects the appearance of a heterotopic cell on day 5, and from 5 to 6 the fact that a homotopic cell on day 5 was no longer recorded, while the heterotopic cell remained. The increase of the across shape DBP
"Remapping" over time: r1029

A) MEAN DISTANCE BETWEEN PEAKS (DBP)
A1) Absolute Mean Values: Within & Across Shape DBP over time

![Graph showing DBP over time](image)

A2) Within Shape DBP/Across Shape DBP Ratio over time

![Graph showing DBP ratio over time](image)

No significant correlation
Linear: p>0.18
Log.: p>0.24

B) MEAN RATE DIFFERENCES
B1) Absolute Mean Values: Within & Across Shape Rate differences over time

![Graph showing Rate over time](image)

B2) Within Shape Rate difference/Across Shape rate difference Ratio over time

Linear: r = -0.78, p<0.05
Log.: r = -0.78, p<0.05

C) Rate Within/Across ratio Vs Peak Distance Within/Across ratio

![Scatter plot](image)

No significant correlation
p>0.74

Figure 9.1. Graphs and Scatter plot depicting various relationships associated with "remapping" over time in animal r1029 of Experiment 2.
measure over time is dramatic, given the paucity of data, and the linear correlation is significant (one-tailed: \( r = 0.82, F(1, 4) = 8.01, p<0.025 \)). The within DBP remains remarkably constant (the entire range of values is encompassed by 6 pixels), and obviously shows no linear correlation with time (two-tailed, \( p>0.55 \)). These results are expected, but perhaps not that meaningful. This is especially so, because the mean DBP w/a ratio (Figure 91:A2) does not show a logarithmic or linear decline over time (one-tailed: linear: \( p=0.18 \); logarithmic: \( p>0.24 \)).

Part B1 of Figure 91 shows the expected pattern. The rate within measure (brown) remains roughly constant (dropping slightly), while the rate across measure (blue) increases. The rate within measure shows no significant relationship with time (two-tailed: \( p>0.16 \)). The rate across measure shows a clear positive correlation with time (one-tailed: \( r = 0.78, F(1, 4) = 6.42, p<0.05 \)).

The mean rate w/a ratio (Figure 91:A2) shows a similar logarithmic and linear decline over time (one-tailed: linear: \( r = -0.58, p<0.005 \); logarithmic: \( r = -0.75, p<0.001 \), see Table 9.2 for F values etc.)

It should be said that since animal r1029 was only taken to day 6, that it is perhaps not surprising that the remapping curve does not show a better logarithmic, than linear, fit.

Part C) of Figure 91 shows there is no correlation between both daily ratios (two-tailed; \( p>0.74 \)).
In summary, if we ignore the significant linear increase of the DBP across measure, then rl029 shows clear remapping based on rate differences only.

**Animal rl077**

Figure 9m presents the data associated with remapping for rl077. Generally, this rat shows the opposite result to that obtained from rl029.

Figure 9m, part A1) shows an expected increase in the across DBP measure over time together with a decrease in the within DBP measure over time. Neither of these relationships is significant. For the across DBP measure correlation, (one-tailed) \( p > 0.10 \); for the within DBP measure correlation, (two-tailed) \( p > 0.18 \).

Part A2), as might be predicted from A1), shows a decline (albeit somewhat messy) in the mean DBP w/a ratio over time. The mean DBP w/a ratio (Figure 9m:A2) shows a more logarithmic than linear decline over time (one-tailed: linear: \( r = -0.50, p < 0.025 \); logarithmic: \( r = -0.59, p < 0.001 \), see Table 9.2 for F values etc.)

Parts B1) and B2) of Figure 9m show no obvious signs of rate difference remapping, and curves look noisier. (rl077 was the animal for which it was least possible to follow many cells over days, though this is not necessarily associated with the cause of the noisiness.) The rate across measure increases slightly with time, but the correlation is not significant (one-tailed, \( p > 0.38 \). The rate within measure is basically constant against time (two-tailed, \( p > 0.99 \). The Rate w/a ratio (see B2)) shows a decline against time, but again the correlation is not significant, (one-tailed: linear \( p > 0.22 \), logarithmic, \( p > 0.17 \).
"Remapping" over time: r1077

A) MEAN DISTANCE BETWEEN PEAKS (DBP)
A1) Absolute Mean Values: Within & Across Shape DBP over time

B) MEAN RATE DIFFERENCES
B1) Absolute Mean Values: Within & Across Shape Rate differences over time

C) Rate Within/Across ratio Vs Peak Distance Within/Across ratio

Figure 9m. Graphs and Scatter plot depicting various relationships associated with "remapping" over time in animal r1077 of Experiment 2.
Part C of Figure 9m, like the equivalent for r1029, shows there is no correlation between both of the mean daily ratios (two-tailed; p> 0.74).

In summary, all of the animals show a decline in both the Within/Across ratios over time, but the correlation with time of this decline is not significant (linear and log.) for the DBP w/a ratio of r1029, and the Rate w/a ratio for r1077, and the decline cannot thus be described as incremental for these ratios in these animals. The decline in the other four w/a ratios was correlated (linear and log.) with time, and thus incremental with time.

**Discussion - Incremental remapping**

Considered singly, r1079 showed both kinds of incremental remapping, r1029 showed rate difference incremental remapping, and r1077 showed peak position incremental remapping.

The W/A ratio is a number whose lowest theoretical limit (with infinite remapping) is 0. (The lowest practical limit is likely to be a little higher - it is likely that even in situations of complete remapping, some cells will by chance be similar, as the Monte Carlo simulation suggests). Accordingly, it is to be expected that the W/A ratio decline with experience will probably not be linear but logarithmic. This is surely the case if the cell population ever reaches the "behavioral steady state" (Quirk et al, 1992). For three of the four sample mean W/A ratios which showed significant decline with experience, the logarithmic fit was better. The exception, the Rate W/A ratio in r1029, is fairly easily explained. It is easy to imagine that one cell from the
sample from r1029 on day 6, had the sample been larger, might have been homotopic. Then it would have been necessary to keep recording for another few days until either the cell remapped, or was not recorded from; then the Rate W/A ratio decline curve would show a better logarithmic than linear fit to experience. Both the linear and logarithmic fits give the same “r” value in part simply because the time-series component was finished quite quickly with this animal.

At any rate, there is no great aim here to say that the remapping curves are more like one or the other model. As we are dealing with still relatively small numbers of cells, with various kinds of sampling and measurement errors, and not thousands of bacteria, there is not much chance of distinguishing between a linear decline that happens fairly quickly, then ceases, and a steady logarithmic decline, at this timescale. The main point is that both a straight line and a logarithmic line represent, applied to our indices of similar/dissimilar firing over time, mathematical abstractions of incremental change. We could equally have fitted logarithmic curves to the combined data of experiment 2, with probably similar “r” and “p” values, but that does not greatly matter. In these circumstances, a straight line is a model of a system that changes in a series of steps. That is unambiguous.

What the straight lines, and logarithmic lines cannot tell us is to what extent each individual cell changes incrementally. Evidence has been presented that this can occur, but is by no means obligatory. Where a straight line based on a population mean is a model of changes, this can occur with many different cells changing in a single step, and remaining stable. Evidence has been presented that this can occur too, if a threshold of firing is applied, so that there are only two categories (“firing” and
“silent”). (For a reminder of both processes, see Figures 9c through to 9f.) It must also be quickly said that the incremental changes must tend to occur in one direction of the dimension, and that the system has stability, or the ability to store changes cumulatively. Allowing for some fluctuations related to sampling errors and measurement errors, this is also the case for the data presented here.

Good evidence, then, has been presented for incremental increase in remapping with experience. It was noted that 2 out of 6 w/a ratios did not show such a relationship. It is in my view no coincidence that the animal from which most cells were recorded (r1079: mean 12.6 cells a day) showed correlated declines with experience for both of the W/A ratios. Because no single measure of remapping was used, a good sample of cells is required for these measures, and this number should be maintained over all the sampled days.

Imagine, as worked out by Muller on the basis of several studies, that “in any given environment, about half the pyramidal cells...are silent” (Muller, 1996 (Thompson and Best, 1989 suggests a larger figure)). Even if the number of cells is constant over days but is only say 8 per day, there is scope for recording primarily monotopic cells on one day, and primarily heterotopic cells on another day, if the analysed sample changes from one day to the next, and the behavioural situation is “Not-quite-remapped”. Stability certainly helps to keep the fluctuations in the sample down, but there is still measurement fluctuation. (Yesterday’s well isolated cell has to be rejected today, and vice versa. In some cases, yesterday’s reasonable tetrode has to be completely rejected today). If, say, 5 out of 8 cells have remapped, on one day, and the remapping cell composition is 4 clearly monotopic, 1 heterotopic, and the next
day it is 6 out of 8 cells remapped, but 4 are heterotopic, and only 2 are monotopic, it will be hard to see the gradual evolution of remapping as a whole using one index for rate, and one for peak position. Remember that no peak position w/a measures are available for monotopic cells.

In spite of these problems, most of the numerous analyses presented in experiment 2 showed incremental remapping.

4) Morph Transfer trials

Introduction

The morph transfer trials were conducted with two aims in mind. The most important question was whether the firing patterns seen in initial exposures to the morph square and morph circle would be different. Can the hippocampal representation of these two environments be modified as a result of previous experience? To what extent does memory affect current representations? Second, if the remapping seen in this study was based, partly at least, on geometry, would the firing patterns associated with a given shape “crossover” to another shape made of different material? Would firing patterns show generalisation?

Procedure

The morph transfer phase basically consisted of recording from the animals during their initial exposures to the morph circle and morph square. The morph transfer phase was conducted on the next recording day after DayEnd, and was thus on Day 7 for r1029, Day 22 for 1079, and Day 23 for 1077 (no recording was done on day 22.). These trials were recorded against a background of baseline trials in the wooden circle
box and wooden square box on the same day. This phase was not completely standardised across the three animals. Animal rl029 was given 4 morph trials (2 in each shape, alternating, morph circle first) interspersed among 5 baseline trials. Animal rl079 was given 2 morph trials (1 in each shape, morph circle first) interspersed among 6 baseline trials. Animal rl077 was given 2 morph trials (1 in each shape, morph circle first) interspersed among 4 baseline trials. Moreover, rl077 had been given one initial morph circle trial at the end of day 21. The data from rl077’s initial morph circle trial was similar to its later morph circle trial, and is ignored in what follows. But it may be appreciated that the later morph circle trial’s novelty was accordingly reduced.

**Results**

This crucial section of the thesis is not quantitative, and therefore all cells from all animals will be shown in the figures of firing rate maps. Figures 9n, 9o, and 9p show the Morph Transfer tests for animal rl029, rl077, and rl079 respectively. The Figures for the Morph Transfer Phase of each animal present the data in a standard way: firing in the wooden circle is shown first, then in the morph circle, then in the wooden square, then in the morph square. In total 40 cells are shown in the three figures.

The results show that two kinds of basic pattern:

a) good transfer from the wooden to morph box in both shapes; or

b) a good transfer from the wooden square to morph square, and remapping from wooden circle to morph circle.

Figure 9n shows the data for rl029 (5 cells). It can be seen that for 4 out of 5 cells, the firing in the morph shapes mimics the patterns seen in the wooden shapes. This is
Morph Transfer test - r1029

Figure 9n. Firing rate maps for five cells from r1029 in the morph transfer phase of the experiment.

For r1029, four morph trials were run, two in each morph box. The morph circle trial shown was the second of the two morph circle trials. The morph square trial shown was the first of two morph square trials. The morph trials shown were used the same morph box.

The figure shows good shape-specific pattern transfer from wooden box to morph box. One cell, cell 4, stops firing in the morph box.
Figure 9o. Firing rate maps for 15 cells from r1077 in the morph transfer phase of the experiment.

The majority of cells show square-square shape generalisation, but only two cells show circle-circle shape generalisation (cells 3 and 10).
Figure 9p. Firing rate maps for 20 cells from r1079 in the morph transfer phase of the experiment.

Most cells show square-square shape generalisation. A minority of cells show circle-circle shape generalisation.
true for the majority of cells that fire in the circle (3 out of 4). Only one cell firing in the wooden circle (cell 4) becomes silent in the morph circle.

Figure 9o shows the data for r1077 (15 cells). It is immediately obvious that the patterns are more varied than for r1029, as might be expected by recording more cells. However, there is an important difference. Only 2 out of 12 cells that fire in the circle fire in the same way in the morph circle (for examples of similar firing see cells 3, and 10). Several cells firing in the circle become silent in the morph circle (eg. cells 2, 7, 9). This kind of pattern is not true of the square-morph-square relationships, however, where the morph square patterns are generally similar to the square patterns (eg. cells 1 to 6).

One cell fires in the morph circle only (cell 14), and one cell fires in the morph square only (cell 13).

Figure 9p shows the data for r1079 (20 cells). The data are similar to r1077. Again the square morph patterns are similar to those of the square (eg. 7, 8, 9), while the circle morph patterns are generally dissimilar to those of the circle. Again this dissimilarity is largely because cells firing in the circle are silent in the morph circle (eg cells 8, 10, 20). It seems that one cell not firing in the circle fires in the morph circle (cell 16), and so for the square-morph-square (cell 4), but these cells do fire on one other trial in the appropriate shapes (data not shown). Finally, while cell 5 may appear to be possibly dissimilar across the square and morph square, this is undoubtedly a sampling error, and on the other two baseline square trials the field is extremely similar to that seen in the morph square.
Results summary - Wooden-to-Morph within-shape firing patterns

Having seen all three figures, Figures 9n, 9o, and 9p the data can be considered as a whole. This is done semi-quantitatively, from impressions based on the firing rate maps. It will not be necessary to agree with all the judgements to appreciate the main points.

The square to morph square patterns show clear generalisation, in that the morph square firing resembles the square firing. Of the 24 cells which fire in the wooden square, 23 show similar fields in the morph square, and 1 does not fire in the morph square. One cell showed very clear remapping (Figure 9o - cell 13), firing (with a 7.1 Hz peak) in the morph square only.

Some cells show clear generalisation in the circle to morph circle patterns but not many. Of the 33 cells which fire in the wooden circle, a maximum of 9 look more or less similar. The dominant circle to morph circle shape pattern shows remapping. Of the 33 cells firing in the wooden circle, 16 shut off in the morph circle, 5 look heterotopic, and 3 firing in both are hard to classify. There are 19 cells firing in the circle morph, 1 of which does not fire on any of the wooden circle trials.

Results summary - Morph square vs Morph circle

Arguably the most important aspect of the results can be seen in a single Figure. This is Figure 9q, which shows 32 cells in the morph circle and morph square. Figure 9q takes a subset of the 40 cells shown over Figures 9n, 9o, and 9p, namely those which
All cells firing in the morph boxes during the morph transfer test

Figure 9q. Firing rate maps for 32 cells firing in either, or both of, the morph shapes during the morph transfer test.

The figure is arranged vertically: cells firing only in morph square are shown in first two rows, cells firing in morph circle are shown in middle row, cells firing in both shapes are shown in last two rows. The order for cells firing in both shapes is roughly from dissimilar to similar.
fired at 1.0 Hz or above in either or both of the morph boxes, and deletes the trials in
the wooden shapes.

Figure 9q shows initial exposures to both morph square and morph circle. The Figure
is arranged such that cells showing similar firing are shown in the bottom row.
Arguably, only 5 out of 32 cells show more or less homotopic patterns across the two
shapes. It is important to compare Figure 9q with the figures of firing rate maps from
Day 1 of experiment 1A and 1B (See Figures 8a, 8b, 8c, and 8d). The morph transfer
phase in experiment 2 is a similar experience to day 1 of experiments 1A and 1B in
that both morph shapes are presented for the first time together, and a very similar
experience to the first two trials of day 1 of experiment 1A in particular (see Figures
8a and 8b). Yet the differences in results could hardly be more striking. What has
happened? How is it that the across-shape patterns are so different in Figure 9q and so
similar in Figures 8a-d?

Discussion - morph transfer phase - information transfer?
The answer, I would argue, resides in the idea that the animals are showing a transfer
of information from one previously-experienced context to another.

First, however, we consider a possible explanation of the fact that many cells firing in
the wooden circle did not fire in the morph circle. Given that the morph circle trials
were given first, it could be argued that the results arise simply out of the novelty of
the material of the morph box itself, that novelty suppressed firing, and that had a
second morph circle trial been given, generalisation might have been seen. While this
could account for part of the pattern seen, I doubt it can explain all the data, and the following tries to argue this case.

1) For r1077, although the morph circle was the first morph box trial on the day shown in Figure 9o, the animal had previously been exposed to the morph circle two days previously.

2) The animal that showed most generalisation of the circle pattern (r1029) showed this immediately on the first morph circle trial. Of three cells firing in the first wooden circle trial, two fired in the first morph circle trial, one at the same rate, the other at a higher rate.

3) Non-circle-shape generalisation did not only take the form of cells shutting off. 5 cells showed heterotopic patterns across the circle shapes. 1 cell fired in the morph circle (peak rate 7.5 Hz) which did not fire on any of the wooden circle trials.

4) Overall, the field peak rates in the wooden square and morph square are rather similar. It is not likely in my view, that the novelty of the morph box could have such a strong effect on the morph circle and yet not affect the morph square at all.

Note that none of these arguments is put forward to deny the possibility that, with more experience in the morph box, more place cell activity would have been seen in the morph circle. Indeed, it may be suspected that in general over time, more and more cells code for a particular environment.

It is patent that the experience of the shapes in the times-series experiment has brought about the dissimilar morph shape firing. It appears that incidentally learned information has been used to bring this about. It is easy to argue this for the retrieval of the square “map” in the morph square, and for those minority of circle cells firing
similarly in the morph circle. Some might argue that this merely suggests a lack of discriminative ability. Certainly, the square appears to be a very salient shape. I take the simple view that an appropriate information retrieval took place.

Others might argue that a genuine knowledge-transfer system would result in four distinct representations, one for every shape/material combination. What would a failure of knowledge-transfer look like? Perhaps a new distinct representation for the morph boxes, but one that was homotopic across them?

Regarding each interpretation of best knowledge transfer, neither the direct copy of information from one shape to the same shape, nor the distinct representation for each box occurred. The result was a mixture of the two extremes. One might simply say that since a mixture of the two extremes took place, at least one of the patterns showed clear evidence of the given interpreter’s chosen test! It does seem clear that further study of this phenomenon is required.

5) Delay Phase

Introduction to analysis

The purpose of the Delay trials was to see what would happen to the firing patterns after a substantial delay away from the testing environment. Would the remapping patterns show stability? This was a test of long-term memory. After the delay, animals were retested in the original setup, in the wooden boxes, and given four trials, two in each shaped box, alternating as normal.
Table 9.3 Table showing number of cells recorded from each animal on Day 1, DayEnd, and the Delay Day, of experiment 2. The table also shows the proportion of the total number of cells contributed by each animal on each of these days.

<table>
<thead>
<tr>
<th>Rat</th>
<th>r1079</th>
<th>r1077</th>
<th>r1029</th>
<th>Total cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td>8 (44%)</td>
<td>7 (39%)</td>
<td>3 (17%)</td>
<td>18</td>
</tr>
<tr>
<td><strong>DayEnd</strong></td>
<td>22 (56%)</td>
<td>11 (28%)</td>
<td>6 (15%)</td>
<td>39</td>
</tr>
<tr>
<td><strong>Delay Day</strong></td>
<td>13 (41%)</td>
<td>11 (34%)</td>
<td>8 (25%)</td>
<td>39</td>
</tr>
</tbody>
</table>
Figure 9r. Firing rate maps for 8 cells from delay test for r1029. The delay was 28 days.

Trials shown are the last two of four trials.
Figure 9s. Firing rate maps for 13 cells from delay test for r1079. All four trials are shown.
Figure 9t Firing rate maps from 11 cells in first two trials of the Delay test for r1077.
Delay test -
The entire sample of cells from all three animals

<table>
<thead>
<tr>
<th>R1077</th>
<th>R1029</th>
</tr>
</thead>
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<tr>
<td></td>
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<td>4.5</td>
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</table>

Figure 9u. Firing rate maps from all cells, from all three animals in delay test of experiment 2. Delays were as follows: from left to right, 1077= 39 days, 1029= 28 days, 1079= 17 days. All these firing rate maps can also be seen in Figures 9r, 9s, 9t.
Figures showing firing rate maps for all the cells were made. For the quantitative analysis, 3 time points were compared: Day 1, DayEnd, and the Delay Day. Table 9.3 shows the number of cells recorded from each animal on each of these days. Since the proportion of cells each animal contributed to the total on these days did not greatly vary (see Table 9.3) it was reasonable to combine the data. This is an important consideration: one wants to increase the numbers so as to give the statistical tests the best possible chance of detecting any possible differences between the DayEnd, and the Delay Day. A one-way Anova was performed with three planned contrasts, between Day1 and DayEnd (one-tailed, expecting lower DayEnd means), between Day 1 and Delay Day (one-tailed, expecting lower Delay Day means), and between DayEnd and Delay Day (no a priori prediction, thus two-tailed).

Results

39 cells from the three 3 animals contributed to the analysis of the Delay Phase. Firing rate maps from all the cells are shown in Figures 9r, 9s, and 9t. Figure 9r shows the delay test data for r1029, Figure 9s shows the delay test data for r1079 (for all four trials - this figure will be compared with a later figure), and Figure 9t shows the data for r1077. The reader may prefer to look at Figure 9u, where two representative trials are shown from the delay test for the cells from all the animals. However cursory the inspection, it will be obvious that the patterns are highly remapped.

The DBP w/a ratios data were as follows: On Day 1 the mean of 14 cells was 1.10 (0.12 sem), on DayEnd the mean of 21 cells was 0.46 (0.08 sem), and on the Delay Day the mean of 17 cells was 0.50 (0.12 sem).
The Rate w/a ratios data were as follows: On Day 1 the mean of 18 cells was 1.02 (0.10 sem), on DayEnd the mean of 39 cells was 0.61 (0.07 sem), and on the Delay Day the mean of 39 cells was 0.55 (0.09 sem).

Anova showed a significant between-groups difference for both the w/a ratios. The DBP between-groups results were $F(2, 49) = 9.94, p<0.0005$. The Rate between-groups results were $F(2, 86) = 6.44, p<0.0025$.

The contrasts were as follows for the DBP w/a ratios. For Day 1 vs DayEnd, the DayEnd means were significantly lower ($t = 4.15, p<0.00025$). For Day 1 vs Delay Day, the Delay Day means were significantly lower ($t = 3.71, p<0.001$). For DayEnd vs Delay Day, the means were not significantly different ($t = 0.29, p>0.78$).

The contrasts were as follows for the Rate w/a ratios. For Day 1 vs DayEnd, the DayEnd means were significantly lower ($t = 3.06, p<0.0025$). For Day 1 vs Delay Day, the Delay Day means were significantly lower ($t = 3.43, p<0.001$). For DayEnd vs Delay Day, the means were not significantly different ($t = 0.59, p>0.59$).

These results clearly show no appreciable differences between the amount of remapping seen in the delay test and at the end of the time-series experiment. There is nothing to suggest that remapping of environments should increase with the passage of significant periods of time without experience in the actual environments. Indeed, there was evidence from one animal in experiment 1 that it does not. (In Chapter 8 we
saw that rl062, tested after a 32 day delay period, was retested in the original setup and showed no evidence of remapping - see Figure 8j for a reminder if necessary.

Accordingly, it seems very reasonable to assume that the maintained levels of remapping reflect long-term memory storage and retrieval. This is a clear demonstration of long-term memory from a hippocampal unit-recording study. It may be the first such convincing demonstration. Before this issue is discussed there is one more piece of data to be presented.

I suspected that after all the experience in the time-series experiment, and for rl029 in quite a lot of probe trials, the patterns after a month or so would be similar to those at the end of the time-series. Could the persistence of remapping patterns be due to the extensive experience of the animals in the environments over many days? To address this question, rl079 was tested again, after a second delay period, with hardly any intervening experience, after the first delay test. Three days after the first delay test of rl079, he was given a one-day series of 7 trials involving various configurations of the morph box, and then another delay, of 29 days, during which he was kept in his home cage. As with the first delay tests, he was retested in the original wooden environments, and given four trials, alternating each shape.

Only 10 cells were recorded during the second delay test. All the firing rate maps for the 10 cells for all 4 trials are shown in Figure 9v. Figure 9v may be compared with the previous delay test for rl079 depicted in Figure 9s, showing the firing rate maps for 13 cells for all 4 trials. With only one animal and not that large a group of cells, no quantitative tests have been performed. In the second delay test (Figure 9v), perhaps
<table>
<thead>
<tr>
<th>Cell</th>
<th>1st</th>
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<td>2</td>
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<td>8.4</td>
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<tr>
<td>3</td>
<td>10.0</td>
<td>0.2</td>
<td>3.8</td>
<td>0.7</td>
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<td>0.2</td>
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Figure 9v. 10 firing rate maps from all trials for all 10 cells from 2nd delay test of r1079.
half of the cells showed similar patterns across the square and circle (cells 6 to 10). In
the first delay test, perhaps 3 or 4 cells out of 13 showed similar patterns, as judged by
eye (see cells 1 to 4). This might suggest decay. In my view, the data do not really
permit a firm conclusion one way or the other. What does seem relatively certain is
that rapid forgetting in the absence of experience did not occur.

Discussion - long-term storage and retrieval

"It is to be hoped that future studies of place cells will help us decipher not only
exactly what these cells encode, and how they do it, but also how long they do it

It is possible that the present results give the first unambiguous, unit-recording,
evidence of long-term storage in hippocampus: altered firing patterns, which, simply
interpreted, reflected learning, were stable for a month. (In fact, the present author is
not aware of any such evidence in any other brain area, but perhaps there is some data
in the monkey literature pertaining to this issue.) For a delay of around 28 days (17
days: r1079, 28 days: r1029, 39 days: r1077), there was no detectable difference in
either direction between the DayEnd and Delay Day levels of remapping.

This is a particularly important result in my view because it was not obviously easy to
predict the result on the basis of current knowledge and theory. One might suspect
that rats have a far-more-limited capacity to manipulate stored material, to, in effect,
mentally revisit experienced environments, when they are outside those environments
(whether in the various kinds of sleep, quiet wakefulness, and so on.) Accordingly, it
might be expected that the storage of patterns associated with the environments might
show appreciable decay, even after a month, especially given the fact that there is no
ostensible reward for having distinct firing patterns. On the other hand, if there is an
important role for consolidation processes in certain neural states, such as slow-wave
sleep and LIA, as has been claimed since Marr (eg. Buzsaki, 1989; Marr, 1971;
Sutherland and McNaughton, 2000), then presumably there is no obvious reason to
expect decay, especially since the two environments and the laboratory testing space
represented such a huge proportion of the rats' experience that was outside the home
cage. Some of the processes understood by consolidation might be able to amplify the
gain of remapping, so long as some remapping has taken place. Finally, the
relationship between LTP and memory is not sufficiently understood to offer a
concrete prediction on whether the level of remapping should be stable or not.

To my knowledge, there has arguably only been one previous unit-recording
demonstration of long-term memory in the hippocampus, and this was limited to a
few cells only. Best and Thompson (1989) recorded a few cells over quite long
periods, including gaps of up to 30 days or so, and showed at least two very clear
examples of cells firing in the same position on a 6-arm radial maze before and after
c.30 day intervals. In my view, this is quite good evidence, but it may reasonably be
argued that there is a fixed-response component to place cell firing (eg. Hartley et al,
2000), and that this stable firing is not mnemonic, or even partly mnemonic. The view
adopted here is that recognition memory was shown by Best and Thompson, and thus
that patterns must have been stable for a previously stored pattern to be recognised,
but this is a debatable interpretation. Of course, such studies say nothing about decay
in the network as a whole. The fact that two cells can be found to be stable does not
Pattern in Octagon resembles that in Circle

1  2  3  4  5

Morph Circle
5.2  1.9  4.1  3.7  0.6

Morph Octagon
3.7  1.0  7.8  9.2  0.6

Morph Square
0.3  0.5  0.8  0.0  6.0

Figure 9w. Firing rate maps from 5 cells from r1029 showing that firing patterns in the morph octagon resemble those seen in the morph circle.

This probe trial series was conducted the day after the morph transfer phase, i.e. day 8.

Although the position of cell 4's field in the octagon may seem somewhat different from that predicted by its field position in the morph circle, it should be noted that this cell's field position is somewhat variable, though always close to the wall. This cell is cell 4 in Figure 9ci.
imply that this is typical, and so on. There is less opportunity for interference, since only one environment was studied. And perhaps the learning is of a simpler kind.

The present study found evidence of some place cells firing in the same place after a delay (data not shown: rl079) but perhaps the more striking finding is that the network pattern, as judged from largish numbers of cells (39 on DayEnd, 32 on Delay Day) remained stably-remapped. It would be of some interest to determine what levels of experience would be required to keep the level of remapping constant (eg. 4 trials a day, once a week?), if it turns out that some decay occurs.

6) Probe trials

After the Morph Transfer Phase, some probe trials were run. Much of the data from the probe trials has not been analysed. What follows is a small selection of examples. In general, probe trial series were conducted with several, often sporadic, breaks. No attempt was made to run the animals continuously as in the time-series experiment, or even very predictably. Quite an extensive series was conducted on r1029, and some of the results may be summarised. The basic idea was to use the morph box in various configurations from day 8 onwards, after the animal had been exposed to morph boxes on day 7.

1) When the animal was placed in the morph octagon, or morph “16-gon”, the cells showed firing patterns in the octagon that were similar to the circle (see Figure 9w). Complete remapping was never seen.

2) When the animal is placed in shapes which were half-one shape (eg half-octagon north) and half another (eg. half-square south) the cells fired in a way predicted by the local space (eg. north circle cells fired in north, and south square cells in south).
Complete remapping was never seen. (An example of this kind of experiment was also obtained from r1079, with similar results.)

These results suggest that in this experiment “remapping” was not arbitrary. In other words, had the main time-series experiment used a rectangle and a triangle, (ie. with no obtuse angles at all), similar results would not have been obtained with the exact same cells. Rather, the cells have, or appear to acquire, tuned responses that are specific to the experienced environments (as, after all, the very clear square-to-morph-square transfer trials showed.) This interpretation is strengthened if one accepts that an octagon is more like a circle to a rat than a square.

Further trials were conducted which relate to this issue more specifically, and perhaps more subtly. Here the aim was to examine cell firing over one broadly similar shape rather than using different-for-rat shapes and comparing firing across different cells. What kind of inputs is the cells’ firing attuned to? Cells which differentiate circle and square may be sensitive to information about the angles made by walls.

For this, advantage was taken of the fact that a particular cell appeared to have a rather constant firing field peak in the morph square. This cell’s field was located tight in the south-west corner of the morph square. Accordingly, the morph square was systematically configured, across a series of trials, into parallelograms with different angles, and the relationship between the peak firing rate, and the angle of the vertex in which its field was located, was noted. This experiment was run over several days, with breaks.
A "Preferred Angle" cell in animal r1029: Cell PA1

This cell shows a remarkably tight relationship between a) the angle of the vertex in which its field is located, and b) firing rate.

This cell was recorded over a 20 day period, from 20/7/99 to 16/8/99, the last day shown on the scatter plot (brown asterisks). It is possible that the cell was "running down" on this last day, on the evidence of the 90 degree baseline trials, and the fact that the cell did not fire on 20/8/99, the next recording day. Systematic manipulation of the morph box into variously angled parallelograms began on 4/8/99.
Another "Preferred Angle" cell in animal r1029: Cell PA2

Figure 9y. Another cell with a "preferred angle", showing a similar relationship between angle and firing rate as seen in cell PA1 of animal r1029. This cell was recorded over one day only, on 20/8/99.
Each day began and ended with the same configuration (the morph as a square, ie with a 90 degree South-West vertex) to act as a baseline. Within this restriction, trials were pseudo-randomised as follows (the angle of the South-West vertex is quoted):

Day 1 (4/8/99) of this kind of trial: 90, 70, 110, 70, 110, 90. The last three trials were run with the raised platform (wooden, not the Trespa platform used in the time-series part of experiment 2 - it was not large enough) rotated 180 degrees.

Day 2 (7 days later): 90, 60, 105, 75, 45, 80, 65, 95, 50, 110, 90.

Day 3 (2 days after day 2): 90, 130, 115, 55, 90.

Day 4 (3 days after day 3): 90, 40, 85, 100, 90.

The aim of Days 3 and 4 was to “fill in” some angles not previously tested, and to go a little further to the extreme ends of the spectrum. In all 13 days spanned the first and last trials, and it is quite remarkable that the cell retained its particular pattern of firing. There is a possibility that the cell was “running down” on the last day. Figure 9x shows the scatter plot resulting from this series of trials. Note that the cell showed similar peak rates when the floor was rotated: see light blue, and dark blue asterisks. (Not shown are data from another day indicating that the cell did not distinguish 180 degree rotations of the morph box, either.) The parametricity of the relationship is very clear. The cell shows a kind of “preferred angle” curve, with a preferred angle in the 60-70 degree range. (The shape of the curve is not unlike that obtained from head-direction cells (Taube et al, 1990).

In summary, it is hard to explain the data except in terms of a sensitivity to geometrical features.
Figure 9y shows this kind of relationship in another cell, recorded over one day, with a field proximal to but not in a vertex. It is worth stating that it should not be thought that all cells with angle and rate relationships show curves with clear peaks - other cells (not shown) recorded simultaneously with the cell in Figure 9x showed more linear correlations between angle and peak firing rate. (This is bound to be the case for some cells, given that many angles (below 40, and above 140, cannot really be tested, at least not for cells with fields in vertices).

Discussion - Probe trials

The technique of using probe trials at the end of an experiment is an important one but is not necessarily always enlightening. In those situations where remapping occurs instantaneously, if one probes the responses of some cells in the remapped state, it is not obvious how such trials could reveal how remapping occurred. The cells would have responses like those of place cells which have just initialised. This problem may be exacerbated by the possibility of pattern completion-type retrieval processes.

The situation may be different in these experiments, because this remapping certainly seems a gradual, learned process, but in future, perhaps one could intersperse probe trials into the time-series as well.

In this study, some cells which began firing in homotopic patterns ended up with monotopic firing patterns. Several kinds of evidence was presented for this, including pictures of firing rate maps.
Cells may begin firing with firing-predispositions, and they may become “tuned” by specific repeated experience. It seems very likely that this second process occurs, but it is not certain whether it occurs against a background of firing predisposition, or random variability.

The finding that circle-specific cells fired in octagons, but not squares, and in locations predicted by their locations in the circle, may suggest remapping based on tuning processes. Thus a cell firing along the edges of the circle may be tuned, inter alia, for inputs relating to walls at obtuse angles, a condition met by the octagon, but not the square.

With much-repeated experience in the two environments, one might expect that cells would tend to become very finely tuned. There is simply not enough data to confirm this, but I suspect it is not the case, and intra-hippocampal connectivity may in part be responsible. Thus a cell that you might expect would require several specific conjoint environmental inputs some of which are not now present in a probe trial may still fire because it is connected to other hippocampal cells where input conditions are satisfied.

A cell was described with a field in a vertex with an apparent “preferred angle” for the vertex. The preference was for acute angles in the range 60-70 degrees. This property was found after the rat had had considerable experience of different morph box shapes, mainly the morph square.
One interesting issue is raised: The animal had had, at most, only one trial with exposure to an acute angle in the morph box. This seems to work against the idea that the cell is matching a template to a previously stored pattern, if it is assumed that the highest rate is produced when the current environmental features most resemble the previously learned environmental configuration.

Returning to the formal nature of the coding, the finding could be explained by inputs relating to distances from walls. But what part of the walls? The finding could be explained by inputs responsive to a specific range of distances to the normal of nearby walls. Other explanations are possible, but unlikely, such as taking the the ratio of the distances between opposing vertices. The given vertex in question is a precise function of this ratio. (In parallelograms with lengths of equal sizes, this relationship is actually well-approximated by a linear function.) Note that a model where the distance between field centres related to firing rates could perhaps account for this, but it is not clear that such a model would be stable, without the updating by actual environmental features such as the angles, distance information emphasised here. It will be interesting to determine how vertex angle-rate relationships are affected in dark trials and so on.

Returning to remapping in particular, the finding suggests these questions:

1) Is this exquisite sensitivity to angle acquired? Is it built upon predisposition?

2) Would we see so many such cells in animals trained only in one environment?

3) If such a cell is seen in animals trained only in squares, will there be a disproportionate cluster of cells with preferred angles of around 90 degrees?
These issues are currently under investigation.
CHAPTER 10:
DISCUSSION

Organization of Discussion

This Discussion chapter examines the following issues in this order:

1) Initial similar across-shape firing
2) Incremental learning
3) Morph transfer phase
4) Delay tests
5) Exploration and environmental discrimination

Five assumptions/predictions of cognitive map theory will be discussed, in passing. In the order of presentation, these relate to: a) incremental learning; b) incidental learning; c) knowledge-transfer d) long-term storage and e) causing the animal to explore.

Similar across-shape firing on initial exposures

Experiments 1A and 1B were designed to formally test the hypothesis that during initial exposures to one walled environment similar in all respects save shape to another previously-experienced walled environment, the firing patterns of hippocampal place cells in both the environments is similar. The hypothesis was not rejected, and indeed appeared to be amply confirmed by the results of Experiment 1A and 1B. While performing many other functions as well, the results of experiment 2, with different apparatus, also confirmed this hypothesis, as far as Day 1 was concerned. At the end of Chapter 8, some mention was made of the ways in which the
hypothesis needs to be clarified and understood. An important aspect of the experiments was the apparently successful attempt to anchor the directional system of the animals, and this stability must be understood in the phrase “similar in all respects”, as well as the fact that the shapes are in the same position in the laboratory testing space.

The Morph Transfer Phase of Experiment 2 also places constraints on the hypothesis. A simple reading of the above hypothesis, seemingly so well confirmed by Experiment 1, cannot account for the sharply, almost totally, different patterns of firing in the morph circle and morph square seen in Figure 9q. The patterns in the morph square should, according to this simple hypothesis, be similar to those in the morph circle, and they are manifestly not similar. So some account has to be taken of the previous experience of the animals in similar shapes, and, in my view, of their experience in the same testing environment.

**The basic finding of initial homotopy: apparent contradiction of earlier results?**

Leaving aside issues implied by the Morph Transfer phase of experiment 2, all the experiments in this thesis flatly contradicted any expectations that firing patterns in circles and squares would be different, *per se*. It seems clear with hindsight that too many assumptions were made in the absence of evidence. This section spends some time on one of the most basic findings in these studies, that circle-square shape remapping is not obligatory. The contention is that our results are probably only apparently in contradiction with the results of earlier studies, but they certainly they would appear to undermine some important previously-held implicit and explicit assumptions based on simple extrapolations from earlier studies.
Diachronic and synchronic approaches: experience and learning in a learning system

It has become obvious since at least Saussure and the post-structuralists that language must be studied at both diachronic (across-times) and synchronic (within-same-time) levels. A sign system should be studied in terms of its successive synchronic states, in order to fully appreciate the meaning of elements in the system, and the interaction between representation and the environment. Applied to this field, neither the single cell studied over time, nor a population of cells simultaneously recorded, are sufficient.

There are very few (published) recording studies that have combined a diachronic and synchronic approach, recording early, middle and later exposures to different environments, or various phases from non-match to sample tasks and so on. (At least preliminarily one need not record the entirety of experience, as was done in this study because the resources were available. It would still have been illuminating to record every 3 days for instance.) It is a little odd that, even in well-resourced labs, where the aim to examine the learning component of cognitive systems is explicit, where it is understood that performance in the given task will improve, where that improved performance process is agreed by others to involve learning, the learning process is ignored: the experimenter records after the learning curve reaches asymptote, i.e. after (most of) the learning has taken place, (and sometimes from cells in an area whose active involvement in that learning task is unknown from lesion or inactivation studies, going on to extrapolate findings as a memory record eg. Wood et al, (2000)).
Of course, it is often simpler to consciously ignore learning, but perhaps techniques and paradigms are sufficiently advanced to begin the process of examining successive synchronous firing episodes now.

The learning issue was explicitly addressed and shelved for later in some of the earlier seminal place cell papers on different environments:

"Before we examine the data, it would be best to first address an important issue. In recording from the hippocampus under varying conditions, the issues of learning, memory, and plasticity are bound to arise. Our purpose, however, was to look for relationships between the environment and place cell firing in the absence of potentially confounding changes in firing associated with learning. [...] In addition to simplifying interpretation of the effects of altering the environment, the reversibility of firing pattern changes simplifies data gathering because the sequence of manipulations is not critical. [...] It should also be noted that this great stability of place cell firing constitutes an excellent baseline from which to look for changes in place cell firing that parallel learning processes." (Muller and Kubie, 1987)

"The rats were intentionally overtrained in this simple task [pellet-chasing], so that cells could be examined in the behavioral steady state, following a presumed initial learning period." (Quirk et al, 1992)

But later, by 1996:

"Firing fields are ...stable regardless of whether the rat spends all its time between sessions in its home cage or some of its time in a different recording
apparatus. If sessions are run in two or more apparatuses over extended times, fields are stable in each.” (Muller, 1996)

An unwritten comment here is that the experiments leading to this conclusion were performed to avoid “confounding changes in firing associated with learning”. When this review was written, O'Keefe and Burgess (1996) had already failed to find the complete or complex remapping between right-angled boxes of varying sizes and aspect ratios. So the dominant view in the field, as represented by Muller’s 1996 review, written with knowledge of the O'Keefe lab results, was that the crux of remapping must be in environments appearing “sufficiently different” (Muller, 1996) to the animals. (Explicit qualifications extended to the fact that different rats might have varying appreciations of sufficient difference.) And so, for instance, Samsonovich and McNaughton’s (1997) model assumed that remapping was always an all-or-none binary switch process. The dominant assumption about the apparent discrepancy between the O'Keefe and Burgess study, finding only what Muller would call “partial remapping”, and the studies of Muller and Kubie (1987), Quirk et al (1992), and Sharp (1997) finding complete remapping, was that the difference between a circle and a square/rectangle (Quirk et al and Sharp = square, Muller and Kubie = rectangle) was appreciably greater than the difference between a square and a rectangle, and sufficient to induce remapping. The concept of sufficient difference is a vitally important one, as will be argued below, but other factors must clearly be considered for a complete model of remapping to emerge, and experience is clearly one such factor.
The results of the present thesis have shown that, in addition to any *a priori* perceptual differences in environments, experience of environments can be a very important factor in inducing remapping. For all the indices of remapping over Days 1 to 6 in the combined data, and for 4 out of 6 indices of remapping over the whole time-series when the rats are considered individually, not only did animals show more remapping at the end of a time-series than at the beginning, but also the amount of experience was correlated with the amount of remapping. (This will issue be discussed further later in this chapter.) Moreover, the experience in the wooden squares and circles produced a situation where remarkably different firing patterns were seen in the morph circle and morph square. This did not occur in experiment 1 during initial exposures to the morph circle and morph square.

It is possible to argue, on the basis of our results, that had O'Keefe and Burgess (1996) recorded for a longer period, they too would have found largely dissimilar across-shape firing patterns in their four shapes, and found that those patterns might not have come about in a single stepchange. It seems that a minority of their cells were already showing patterns which are hard to explain with models of homotopic firing (Hartley et al, 2000). It would be easy for a future study to test the idea, doing the O'Keefe and Burgess task for a couple of weeks, say.

So one could try to explain the discrepancies between the O'Keefe lab experiments and those from the Muller and Sharp lab in terms of levels of training and experience. This account would need to argue that the complete-remapping seen by Muller and others is similar to that seen on day 6 and 7 of r1029 (see eg. bottom part of Figure 9ci) and on later days of the other animals (see eg. Figure 9b for day 18 of r1079). It
would need to argue that the complete-remapping seen in other experiments passed through unrecorded phases of incomplete-remapping. Such an account might also point out that although 2 rats were taken to 21 days in experiment 2, the data by the fourth, fifth and sixth days (with 19, 19 and 12 cells respectively) already showed clear evidence of sharp decline in w/a measures. And though none of the three animals taken to the end of experiment 1B showed complete remapping, such animals might have been slower to remap. The fact of remapping at different rates in such an account could easily be attributable to rat-personality differences, and so on. This account is not unattractive. There are problems with this sufficient-experience-for-learning explanation, however, and it is worth considering these, as well as other explanations.

What does “complete remapping” look like?

Two problems with evaluating the sufficient experience explanation are that we cannot easily compare studies, and that we do not have a good model of what complete remapping ought to look like. The practical issue first: none of the studies (Muller and Kubie, 1987; Quirk et al, 1992; Sharp, 1997) show a complete picture of all the fields recorded, the number of cells recorded from any one animal is generally low, and the comparisons are between obviously different patterns (eg circle-to-circle vs circle-square correlations). One emerges with little sense of the magnitude of the differences. This study is not that different. DayEnd is certainly very different from day 1, but it is it like other groups’ data? The field is in need of good quantitative indications of remapping, but in the meantime perhaps more firing rate maps should be shown.
We may have a pretty good model of random firing for cells that fire in two environments. The Monte Carlo simulations used here were simple and easy to understand. Parameters could be tweaked to take account of cells tending to fire near edges, if necessary, and so on, to get a better idea of probability densities. However, the complication presented by remapping is that we do not know how to take account of cells that are "silent". What is x in a statement of the following form: If the patterns in environment A and B are completely unrelated, then less than x % of the fields firing in one environment should fire in another. While the day 6 and day 7 patterns from r1029 seem to be "completely remapped", do the patterns in the 20 cells from one animal in Figure 9b show complete remapping? Are they comparable to the 18 hippocampal cells (over all animals) in Quirk et al, 1992?

It is difficult to have good answers to these kinds of question, but the sufficient-experience argument requires that the DayEnd patterns should be like those of other studies.

Is the single factor of experience sufficient to account for all the apparent discrepancies with earlier work?

Here it will be argued that the answer to this question is probably "no". Certainly, it is a better explanation of discrepancies with the Muller lab results, than for those obtained by Sharp (1997). Unfortunately, not all the studies mentioned provide sufficient detail to examine the question properly. O'Keefe and Burgess (1996), within the straightjacket of a Nature publication, give no temporal details of training whatsoever. It is unclear from Muller and Kubie (1987) whether the animals received training in the rectangle, but exposure to the circle (in all that follows, all descriptions
of shape assume walled environments) was of the order of 2 weeks, at 15-30 minutes a day. From details in the Quirk et al (1992) paper, the impression seems to be that rats had 10 days experience in the circle, followed by 5 days in the square. Sharp’s paper (1997) is unambiguous: rats received 15 minutes experience per day for 5 days in the circle only, and no training in the square. To all these figures, must be added the time where an animal is repeatedly exposed to the same environments so that more cells can be added to the total recorded. For instance, it is not possible to recover from Sharp’s paper whether any given square trial firing rate map shown represents the first trial in the square. This may well be important.

In experiments 1 and 2, it is possible for the reader to recover the time spent in the environments. There were very few technical problems, and where they occurred, the rat was immediately taken out of the environment.

While the Muller lab training regimes appear to have been quite extensive, this was not so in Sharp’s study. Sharp’s training regime, while longer than for all the animals in the pilot study (except r966) and experiment 1A, was very similar to that used in experiment 1B in this study (training in one shape only, 8-10 trials of 8 minutes each over 3 days), yet the results are utterly different to those of experiment 1B. This is even allowing for the fact that for only 10 of the 20 place cells recorded was there a return-to-baseline condition: all the cells remapped in her study. In my view, the complete remapping obtained by Sharp is related to the nature of the environments and testing space. As the title of the paper states, the two environments in the study were “geometrically and visually distinctive” (Sharp, 1997 [my italics]). Thus, in contrast to the other studies mentioned here, the to-be-distinguished environments
also contained visual patterning differences. It is not unreasonable to assume that these differences are additive, and may even combine non-linearly in the perception of environmental difference. There is also another difference in the environments that is ignored in the paper's interpretation (and the title). Quite simply, the circle and square were in different positions in the room.

The experiments in the pilot study of this thesis clearly showed that where remapping took place, in the two animals which had received more than a bare minimum of exposure to the environments, and in one which had had very little training, it was primarily position that was the basis of the remapping, and not shape (eg. see Figures 7a and 7b.) In combination with different visual patterns, it may be suggested that this difference was very important to the complete-remapping result observed in Sharp's study, and that the single factor of experience is unlikely to have been wholly responsible. At any rate, there are clearly more than enough differences in the experiments of Sharp and this study to say that they are not in conflict, and there are reasonable hypotheses, some based on the results of the pilot study, which can make sense of the differences. The next section considers the studies from the Muller laboratory.

**Muller and Kubie (1987) and Quirk et al (1992): Similar procedures to this study**

In these studies, there were no obvious differences between the remapped environments except shape. Although these studies did not, it seems, consistently use two different versions of each shaped environment, as was done for Experiment 2 here, there is no obvious need to argue that uncontrolled variables in the two environments used, such as smell, could be responsible (eg. additively) for the results
of complete remapping seen in the Muller-lab studies. (A replica circle was used in Muller and Kubie, 1987, but it is not clear that it was used in the circle-rectangle remapping experiments.) Moreover, no evidence whatsoever was seen of cells consistently firing in a unique way in one of two boxes of the same shape in experiment 2. The environments used in Experiment 2 were clearly meant to be copies of those used in the Muller laboratory, even to the white, internal, cue cards.

Muller and Kubie (1987) and Quirk et al (1992): Different procedures to this study

There was one important procedure however, which the present experimenter clung to, that is in a sense opposite to the procedure employed in the Muller laboratory, and I will try to argue that this may well be an important factor, as well as experience, in explaining apparent discrepancies. In other words, on some occasions at least, even if the cell firing had been recorded in initial exposures to the two environments in the Muller studies, it is possible that already-complex-remapping might have been seen. I apologise for the following discussion, which is somewhat laboured and involves comparison of experimental procedures.

It is hard to summarise the procedure in question in a phrase, because that would imply that the phenomenon was understood, but it might be something like "established position-direction constancy". It may be said at the outset that the argument is not purely about head-direction constancy. There is also the issue of position-constancy, which may be hard for the rat to appreciate under certain conditions.
The reader is reminded of procedures in these experiments, and of those employed by Muller and colleagues.

In both Experiments 1 and 2:
1) The rat is brought into the room in a standardised way, and placed on a holding platform. 2) Recording does not commence for about 20 minutes. 3) Between trials, the rat is again placed on the holding platform. 4) The rat is always introduced into the environment with its head facing north, the path of passive displacement always through the south side of the black curtains, and over the south side of the environment. 5) The rat is always placed into the centre of the given environment.

There are no exceptions to this, except in probe trials. By contrast, in the Muller laboratory:
1) There is no holding platform. 2) Between trials, rats are returned to the home cages (presumably in other rooms, but confirmation of this is awaited.) 3) Usually (eg. Bostock et al, 1991; Fenton and Muller, 2000) rats are introduced into the environments through one of four cardinal places in the curtains. (This is the usual procedure if one plans to do rotation experiments, which were done in both Muller and Kubie, and Quirk et al.)

The procedures in the Muller lab are designed precisely so that room position-direction relationships are hard to establish, and thus that the cue card in each of the environments will almost-ideally control the angular location of place fields, when the cue card is rotated. In some laboratories indeed where remapping is studied (eg.
Tanila’s), the rat is purposely disorientated before being introduced into the environments.

In addition to preventing direction-sense constancy, such procedures make it difficult for the rat to know where it is in a wider reference frame.

Established position-direction constancy: “all novelty is but oblivion”

“[A]s Plato had an imagination, that all knowledge was but remembrance; so Salomon giveth his sentence, that all novelty is but oblivion.” (Francis Bacon, Essays).

If it is the case that remapping is obtained in circumstances where position-direction constancy is violated, it is far from certain that the remapping represents learning at all. In these circumstances, remapping may just be a reset-initialisation aspect of the system. (What system? The answer is unclear, because disorientation (using the term broadly) is not well understood, but there is clearly and as yet mysterious interaction between directional and place systems.) The patterns once remapped need to be instantiated, for stability, and for the patterns to be reproduced when the animal next enters the environment, the animal needs to recognise that the environment is similar.

Such non-“position-direction constancy” procedures help to understand the reset-initialization process, and the environmental recognition-memory process. Such procedures, however, do complicate the study of the hippocampal system itself, and probably do not contribute to the study of discrimination learning.
The experiments in this thesis were based on trying to isolate changes in the hippocampal firing system. The firing of hippocampal cells is controlled by the directional system and the place system. To study the place contribution, we need to ensure that the directional system is anchored and unchanging from trial to trial, in a way that is understandable. It is commonly thought that the directional system influences place cell firing more than the other way around, and there are also in my view, unknown ways in which an animal codes for the fact that two environments occur in the same position in a room. Both the known directional coding, and this more speculative room-position coding, may not have been constant in the Muller lab experiments. The rats' directional sense in the Muller lab experiments is almost certainly reset upon entry into the environment, if it is the case that random cue-card positions, and random-entry points are used in rotation trials, and the cue-card controls the field orientation.

The point of the reference to Bacon is that the initialisation of new patterns in one environment may represent "oblivion". No wider-frame constancy has been permitted to emerge: a sensory-like reset occurs in the absence of a stability recognised by memory, and in the absence of an intra-environmental similarity sufficient to retrieve a previous pattern. To the pattern for environment A, there is now a new pattern, which happens to occur in environment B. The crucial point is now that when environment B occurs is re-entered, an intra-environmental recognition memory process occurs. So one ends up with two stable patterns, without discrimination learning as such. In such a model, there is of course a role for sensory discrimination: since the animal in A reproduces the previous A pattern, the animal in B may reproduce pattern A on the basis of the intra-environmental cues, but does not. (In
fact, I would predict that this will sometimes occur.) In the experiments of Kentros et al (1998) showing remapping across geometrically similar but visually distinctive environments, blocking NMDA receptors did not prevent remapping in the novel environment, did not prevent the old previously-experienced-without-NMDA-R-blockers environment pattern being reestablished, and only prevented the first-exposure pattern in the novel environment being reestablished on later exposures. This suggests that neither the remapping process, nor the recognition memory process requires NMDA type learning.

So there are two factors that need to be built into a remapping model. One is the idea that the environments be sufficiently different, the other is that they be sufficiently experienced. I have tried to argue that the “sufficiently different” part of the model of remapping must not pay attention simply to the intra-environmental cues, such as colour, and shape, but also to the wider arena. In situations where apparently complete remapping occurs instantaneously, we may consider there are three processes: reset-initialisation, pattern instantiation, and pattern recognition. Only pattern instantiation may require NMDA-based learning processes. Pattern instantiation should also be taken to include those patterns relating to room position, direction sense and so on.

This section ends with the brief comment that in the study of O’Keefe and Burgess, which did not find “complex remapping”, it is likely, as intended, that the rats were able to establish position-direction constancy.

**Experience as a factor in remapping in this study: incremental learning**
Comparison with other studies has not been a simple matter. Position-direction inconstancy, intra-environmental differences, and experience may all contribute to the varieties of results seen as a whole in this field. It is much easier to be certain about some of the results of this study, which is arguably more standardised and more transparent than most. And perhaps the most certain conclusion of all is that experience played a major role in bringing about the observed remapping. In experiment 2, the DayEnd firing patterns, showed, as predicted, lower scores of across-shape similarity than the Day 1 patterns: the two W/A ratios on DayEnd were about half what they were on Day 1. In all, the outcome was very improbable if the two samples on these days were drawn from one unchanging population.

More than this however, a more detailed version of the hypothesis that remapping would increase with experience was tested; namely that the decline in similar across-shape patterns would specifically correlate with the amount of experience in the environments. This was looked at in several ways. For the same combined data from day 1 to day 6, various types of analysis involving the two W/A ratios, and “homotopic cells” as defined by the CPCP distance, were used. These all showed clear linear correlations between the similar-firing indices and time. For the animals considered individually, four of the six possible sample mean W/A ratios showed good linear and logarithmic declines with experience.

These results appear to be in contrast to those obtained by Bostock et al, (1991) studying remapping in circles with different visual patterns (a black cue card vs white cue card). There is no obvious candidate explanation for this apparent discrepancy. The position-direction constancy argument might be put forward. Since the results of
the Bostock et al (1991) study are similar to those of Kentros et al (1988), it seems possible that the origin of the altered-pattern of firing seen in the two environments is not NMDA-receptor learning based.

The present experimental results offer interesting ways of re-examining assumptions of cognitive map theory. Two issues are raised. Firstly, the cognitive mapping system is assumed to be a one-trial learning system only (basically), and this does not seem, simply interpreted, to capture the shape of the results here. Second, the cognitive mapping system is supposed to be capable of incidental learning (as well as other types of course), and this does seem to capture the results here.

**Incremental learning and the cognitive map theory**

In as much as hippocampal cells provide the basis of the map in the original cognitive map theory (there may be reasons to think the map is elsewhere), the present results may present a challenge to cognitive map theory.

"Unlike the extra-hippocampal systems, the locale system is relatively free from the effects of time and repetition. [...] Incorporation of information about stimuli occurs in a non-incremental fashion. The map itself can become richer and more distinct (i.e., there is better and finer differentiation of places) but it is not altered in any fundamental sense with repeated exposures to the same environment.”

(O'Keefe and Nadel, 1978, p.95).

In my view the present results do not in the least contradict “single-occurrence storage” (ibid, p.384). Furthermore, on the basis of many hundreds of recording
sessions, with many animals, including the very first exposures to environments, it
does seem very likely to the present experimenter that more cells become associated
with an environment. This is hard to prove conclusively, for reasons given earlier
related to absence-of-evidence type issues, but this would tend to support the “better
and finer differentiation” as well.

That incorporation of information into the map occurs in a non-incremental fashion is
not necessarily inconsistent with whole-map incremental remapping if each cell
remaps abruptly. It could be that particular areas within the environment, represented
by particular cells, are recoded, but not simultaneously. A further confound is that, as
we have seen in Figure 8iII, two cells with fields in similar regions of both shapes
may have rather different underlying input components. In which case, to show the
the cognitive map position is incorrect requires rather more detailed knowledge about
the underlying input components to each cell.

What seems hard to avoid, however, is the conclusion that the evolution of monotopic
and heterotopic patterns between two environments with experience, and the change
that this implies in each environment, is rather fundamental. Moreover, evidence was
presented that for at least some cells the transition from similar to remapping firing
took more than one day.

It is possible that the hippocampal system has features of both locale/episodic and
taxon/semantic learning systems (O’Keefe and Nadel, 1978; Tulving, 1984). It will be
important to establish whether the incremental change in the alteration of the
hippocampal firing patterns across different environments reflects incremental
changes in behaviour in a task in such environments which is shown to be hippocampally dependent.

**Incidental (aka. latent) learning and the cognitive map theory**

It is possible to argue that only the simplest kind of incidental learning has been demonstrated previously, in so much as fields form in environments which are not rewarded (reviewed in O'Keefe, 1979). In fact, even this simple incidental learning has not been demonstrated beyond all argument. One perhaps trivial point about the place fields in O'Keefe’s unrewarded environments, is that he did not go on to show that they were stable in the long-term, and thus it is not entirely clear that an NMDA-type pattern stabilization took place after pattern reset-initialization. Those experiments which have showed long-term stability (here meaning anything beyond a couple of days) have always used reward in the recording chamber. In the screening procedure used for these experiments, cell field stability was seen on the unrewarded holding platform, which supports the points made by O’Keefe, 1979, though few trials were recorded. It is of course always hard to argue for motivation-neutrality of an environment. The holding platform was a (relatively) safe haven etc.

But let us assume that the simple pattern-stabilisation kind of incidental learning has been shown. The results of this thesis have shown incidental learning of a more complex kind, that of gradually seeing differences in things that are rather similar in many respects. Is this of interest?

Why does thorough/complete remapping take so long? Is the current task a useful way of investigating hippocampal function?
One analogy of this learning might be as follows. Imagine a student of the Anatomy Department of UCL, obliged to use the department lift. The task, retrospectively seen, is to differentiate floors, from the view inside the lift. This lift is extremely slow, so the acceleration gives almost no clues. Even with minor reward to differentiate the floors (he will not waste time getting out on the 2\textsuperscript{nd} floor, when he only wishes to go to the 3\textsuperscript{rd} floor), the process may take considerable time. The task can take months, if the learning is incidental; the main occupations in the lift being reading, and talking to colleagues etc. (rice-chasing). Floor discrimination is a common problem with multi-storey car parks. In Perth, Australia, the council has placed many pictures of animals, a given animal unique to each storey, on the walls of each storey, in order to help differentiate the similar-but-different storeys. It seems very possible that the neural representation of each storey, and each floor for the Anatomy student, diverges incrementally.

Certainly, attention is important, but there is no coherent hypothesis which can use attention to explain the current results. If anything, the animal spends less time near the walls over time. And no obvious correlation between rearing number and speed of remapping was seen.

It is not to be doubted that learning might proceed much faster if a car-owner lost her car, or the student were fined, as a result of going to the wrong floor. But this may not be important for our considerations here. Firstly, it is possible the such learning based on punishment and reward is more susceptible to decay. (In a shock place-avoidance task an animal may avoid a place after only two shocks, but revisit it under extinction conditions after an hour or so). Most importantly, however, the speed is not always
the important thing. If information learned slowly confers advantage later, this is better than no learning at all; and there will surely be situations where no obvious benefit is at first associated with knowledge of certain environmental information, but is later. It might take a while to learn incidentally the layout of a complex environment, but when fire blocks 7 of 8 possible routes which lead to safety, knowing the crucial 8th is very useful. If it is the case that some brain systems support this kind of learning, and some do not, then we need to find out which they are.

This argument is perhaps laboured, but it is vital to point out that most tasks use reward and punishment contingencies directly to induce learning. Given the theoretical importance of the idea that a cognitive-mapping hippocampal system uses incidental learning, and this is one of the crucial ways in which it differs from other neural systems, and cannot obviously be modelled by S-R neural networks, then it is clearly unsatisfactory to use reward to induce learning in all experiments. This may be another way in which the present experiments tap into the theorised specificities of the hippocampal system.

In summary, in trying to ensure that altered hippocampal patterns are not simply passive reflections of input from other regions (eg presubiclar directional system), and in keeping to a task where discriminatory responses are not rewarded, the present task may be a useful mining tool for extracting hippocampal-specific function. The obvious weakness is the lack of a behavioural link. Future work should focus in my view, on testing behaviour after incidental learning processes, and less on exploring cell responses simultaneously with overtly rewarded responses.
One experiment of the kind that tests behaviour after incidental learning process might go as follows. 10 rats are given equal amounts of experience in the morph circle and morph square, with 100 cells recorded from each. Each rat shows a different amount of remapping. At the end of the allotted time for remapping, entry into the north east region of the morph circle elicits shock. Entry into the north east region of the morph square does not elicit shock. There is food there. At first, presumably, all rats avoid the north-east region of the morph square and morph circle. However, do the rats that have remapped the most begin to go to north-east region of the morph square sooner than those which have remapped the least? Is there a correlation?

The Morph Transfer Phase and incidental learning: evidence for “knowledge transfer” based on “latent learning” in a “cognitive map”-type system?

The results in the Morph Transfer Phase may be the hardest of all to interpret to the satisfaction of all. The view taken here is that these results extend the notions of incidental learning built up so far in the Discussion, and show what might be summarised as the knowledge-transfer process predicted by cognitive map theory, and also other theories of hippocampal function. The basic argument advanced in Chapter 9 was that information gained in one context is used in another. One could imagine a version of the above imagined experiment, where the environments used in the specific-region-shock phase are not exactly those used in training.

Long-term hippocampal storage: The Delay Tests

Long-term storage is one of the predictions of cognitive map theory:
"The hippocampus...both constructs and stores cognitive maps. [...] The hippocampus is involved in at least the long-term storage of place information."

(O'Keefe and Nadel, 1978, p.374, 377)

This issue has become an extremely vexed and controversial one. There has been much blood-letting relating to the old and classic debates on consolidation, retrograde amnesia gradients, and more recently whether hippocampal-lesioned patients can have remote topographical map memory (eg. Nadel and Moscovitch papers, Teng and Squire, 1998). Of course, the implications of the present study are not easily applicable to long-living humans with an extended capacity for the manipulation of information outside of the context in which it was gathered.

In chapter 9, it was argued that the delay test extended the way in which the present study was an examination of learning and memory. The present thesis contributes information to all the components of presumed learning and memory processes except the theorised role for consolidation occurring outside the environment: learning itself, (incremental, and incidental), storage, and retrieval (recognition, not recall) in the "short term" and "long term" (at least as applied to animal studies). In as much as it was possible to falsify the long-term memory assumption about the cognitive map theory with this simple delay test, it was not falsified.

**The role of the hippocampus in exploration: environmental discrimination**

Some of the more descriptive, impressionistic results from the experiments have been left to this part of the Discussion. Here we are concerned with behaviours such as rearing, wall-climbing, and general thigmotaxis. The discussion here relates to
cognitive map theory. One of the most strongly-phrased predictions in the hippocampal cognitive map theory concerned the exclusive role of the hippocampus in exploration:

"The hippocampal locale system is assumed to form the substrate for maps of environments an animal has experienced; these maps are established in the hippocampus during exploration, a species-specific behaviour pattern concerned with the gathering of information. [...] Exploration is a direct response of the animal to the detection of a mismatch by the locale system; in the absence of the hippocampus all forms of exploratory behaviour should disappear from the animal's repertoire." (O'Keefe and Nadel, 1978, p.242; their italics.)

The exclusivity of the hippocampal role is clearly insisted upon. However, novelty may occur in several single modality and cross-modal regions and it is not clear why different brain regions should not be capable of driving apparently-identical motor behaviour (eg. a rearing event.) O'Keefe and Nadel drew a distinction between novelty and noticeability, suggesting that only novel stimulation elicits exploration, while noticeable (ie. exciting) stimuli elicit approach or avoidance behaviour in accordance with their biological meaning. Novelty implies noticeablity but the converse is not that case. There is also an issue with regard to what is understood by exploration. In my view, rearing is good evidence for exploration, but wall-climbing and thigmotaxis may represent approach and avoidance behaviours as well as exploration.
The following impressionistic, but sometimes quantitative, results were seen with changes to environments:

1) In the pilot study, when the standard shape-in-position configurations were altered so that a different morph-shape was created by the experimenter in the same position, although the place cell patterns were basically unaltered, at least two animals appeared to explore the walls more in the first half-minute or so than usual. The record of the paths made by the animals appear to confirm this, in so much as they are more proximal to the walls than was usual.

2) In one probe experiment where a different square was used, although several cells’ firing patterns (but not all) seemed similar to the standard square pattern (data not shown), rearing (unquantified) increased considerably. It must be stressed that there was certainly more difference between the standard-square-to-novel-square than the standard-square-to-standard-square patterns.

3) In experiment 2, rearings were quantified in two animals (rl077 and rl079) during the Morph Transfer Phase day. Rearings were appreciably more numerous in both the morph circle and morph square, as compared to the wooden shapes. This is perhaps the most important observation.

A simple interpretation is that while there is sometimes a parallel relationship between rearings and remapping (Exp 2: the wooden circle - morph circle place cell patterns were substantially different) there is also dissociation between place cell responses and exploration (Exp 2: the wooden square and morph square patterns were very similar; Pilot: the morph square and morph circle patterns in the same position were similar).
There are certainly observable, though usually subtle, differences between the patterns, even where clear "shape-generalisation" was seen, to say that part of the hippocampal code triggering exploration are these subtle differences. This is tenable, but not very convincing with, say, the morph square trials for r1079. More likely, there may be aspects of hippocampal signals that are simply escaping experimenters with current recording techniques and measurement-paradigms.

But the simplest interpretation would assert that extra-hippocampal brain systems are capable of detecting modality-specific some cross-modal, novelties, and such systems can drive the motor systems subserving exploration.

It might be imagined that for some, the sometimes apparent dissociation between rearing and place cell activity might provoke a statement of the form "animals do not need hippocampi for environmental discrimination". But there is an obvious difficulty with the understanding of "environmental discrimination". This concept has been left hanging until now. The phrase "discrimination learning" has been widely used in this Discussion as though it were obvious that this is what the remapping patterns imply. This is not clear, but the first point is that rearings and place cell dissociations do not falsify this implication in themselves, unless one thinks that the rationale behind the aspect of cognitive map theory mentioned above is particularly cogent.

It seems obvious that one could design a test involving two environments, which requires the animal to do one thing in one, and another thing in another. It would not be at all surprising, or even that interesting, if any hippocampal remapping in the two environments lagged behind the discriminatory responses. It is extremely plausible
that the animals could respond to olfactory differences in the environments. If smell x occurs, go left, if y occurs, go right and so on. Such an experiment is only of special interest when factors such as olfaction are controlled for. In my view it is not easy to say exactly what kind of learning the remapping represents, without further study. Carefully controlled experiments testing behaviour in the morph box are needed to tease out the issue of whether remapping represents environmental discrimination in the sense where each environment is a single global context. Such experiments would need to rule out ways in which parietal cortex might also be able to subserve a discrimination.

It remains possible that the evolution of remapping seen in the present experiments represent complex navigation-related differences associated with the two environments. The present experimenter has not seen any obvious ways in which remapping could come about in terms of different routes taken. For instance, imagine that in the circle the animal always moves west through a field, in the square he moves east through it, and eventually the field dies in the circle. No evidence of such patterns have yet emerged from inspection of the data. It is just possible that one needs to look harder, and more cleverly, at this type of issue. There has not been much time for detailed inspection of individual cells.

Finally, it is important to note that the lesion and genetic-manipulation literature on the role of the hippocampus in so-called context discrimination and context conditioning is not very consistent (eg. Frankland et al, 1998; Good and Honey, 1997; Good et al, 1998; Phillips and Ledoux, 1994; and reviews by Holland and Bouton, 1999; Maren et al, 1998). All combinations of positions to hold on hippocampal
involvement are represented by at least one laboratory. Studies suggesting that hippocampus is not involved in context discrimination need to establish clearly that it was indeed something like "context" that the animals used to discriminate the environments in their particular task, and not particulate cues. As for context conditioning, a possibly increasingly influential view (See Phillips and Ledoux, 1994, and Good et al, 1998) is that hippocampus is only involved in so-called "background" contextual conditioning, eg. where the primary or "foreground" association involves an explicit (eg. tone) CS paired with a US. In situations where there is no CS, and thus where contextual cues become so-called "foregrounded", then the conditioning can take place without hippocampus. This is not yet a consensus view, but perhaps is gaining more support. Finally, the relationship between a "context" and an "environment" is unclear. "Context" is sometimes synonymous with "configural cue situation", and there is no particular reason to think that such paradigms tap into hippocampal function.

In summary the behavioural literature does not give us any concrete expectations about the role of hippocampus in environmental discrimination.

The obvious gap in the present experiments is the lack of a behavioural test, such as the one suggested above in the section on incidental learning; where rats showing high levels of remapping across the morph square and morph circle might be more inclined to visit a region in the square whose isomorphic region in the circle elicits shock.
APPENDIX

Examples of firing rate pattern changes in individual cells

This graph shows examples of the four basic kinds of pattern of firing rate changes over time. The x axis represents day. The y axis represents the amount of firing in the shape in which the cell ended up firing in most ("preferred shape"), as a proportion of the total firing in both shapes ("Proportion of firing in preferred shape"). Linear correlations of this preferred shape firing rate proportion against time are shown in the key. The two significant linear fits are indicated in the graph.

Cell a: shows very clear evidence of incremental change.
Cell b: is an example of a cell whose firing rate shows only a mild shape-preference, which is roughly constant over time.
Cell c: shows good evidence of incremental change ($r = 0.78$), but it could be considered that the main changes occur from days 13 to 15.
Cell d: is an example of a monotopic cell which shows a very strong shape-preference from the moment it is recorded to the end of the experiment.

Cell a is from r1029 (shown in Figure 9d), cells b and c (shown in Figure 9e) are from r1077, and cell d is from r1079.
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GLOSSARY

Measures of place cell firing (see Jung et al, 1994; McNaughton et al, 1983; Muller et Kubie, 1989; and Skaggs et al, 1993 for further details eg. equations)

Spatial information content – If not otherwise mentioned spatial information is measured in bits per spike. It is a measure of how much information about spatial location is conveyed by a single impulse of a given cell.

Sparsity – Sparsity is a measure of how diffuse cell firing is in the spatial domain.

If a cell fires equally all over the recording environment, information per spike is 0, and sparsity is 1. If a unit fires evenly over half the environment, and never fires in the other half, the information per spike is 0.5, and the sparsity is 0.5.

Spatial Information rate – This is obtained simply by multiplying the information per spike (see above) by the mean firing rate of the cell.

Spatial specificity – The precise meaning of this term varies a little between various authors but always refers to the size of a place field in comparison to the environment traversed. In McNaughton et al, 1983, the environment was an 8-arm radial arm maze, and arm specificity was defined as the summed firing rate over the arm showing the highest rate, divided by the mean firing rates for the other 7 arms.

Spatial coherence – This measures the extent to which the firing rate in a unit of the environment (a pixel) is predicted by the rates in its neighbours, and therefore estimates the local orderliness of the spatial firing pattern.
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