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Integration of geometric and contextual inputs to hippocampal place cells

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I would like to dedicate this thesis to my Nan, Elisabeth Norman, without whom none of this would have been possible.

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Abstract

Neurons in the rodent hippocampus fire in highly restricted portions of an environment. These place cells have receptive fields called place fields and are argued to form a representation of space. The work described in this thesis explores the different types of sensory input to these cells, how these inputs are integrated and the implications for our understanding of hippocampal processing. To this end, hippocampal pyramidal neurons were recorded from awake, behaving rats as they foraged for food in a series of different environments. By manipulating the environments to which rats were exposed the nature of the input to place cells was elucidated. The first two experiments explored the influence of geometry on place fields. A novel environment was created that facilitated an examination of how the boundaries that constituted that environment affected place field activity. It was found that the presence of boundaries was important in order to have well-defined and consistent place fields across trials. Furthermore, exposure to one environment affected the place fields recorded in a similar but different environment, suggesting that learning was occurring. The final experiment examined in greater detail the effect of learning on the place cell representation. Place cells were recorded in two neighbouring environments that were the same colour. Initially similar place cell representations were found to diverge over the course of several days and weeks such that the place cell activations in both environments became distinct. Once a distinct pattern of place cell activity was seen, the

colour of the environments was changed. The learnt discrimination that was acquired in the initial environments was not transferred to the novel environment. This suggested that the information acquired by place cells was specific to a given environment. These results are incorporated into, and extend, an existing model of place field formation.

1. General Introduction

1.1. Overview

The mechanisms and processes by which we locate ourselves in the world have undergone extensive characterisation. Questions about the space around us and our relationship to that space have interested philosophers and scientists for thousands of years, from Aristotle to Leibniz and Newton. In the last 50 years our knowledge of the neural systems that mediate functions such as accurate self-localisation, path-finding and navigation has exploded. Evidence from humans with specific lesions of proscribed brain areas, extensive work in many different animal models and computational and theoretical accounts have helped elucidate these processes. One of the questions addressed in this thesis relates to how a brain is able to compute an accurate representation of location based on its immediate surrounding environment. Specifically, what information is provided by the boundaries and/or barriers that define an environment? Another question is what specifies a location or place as unique from another, similar location? Using techniques that allow the recording of neurons in the brains of awake, behaving animals has allowed a close examination of the microstructure of the representations that mediate these functions.

The work described in this thesis examined the microstructure of the hippocampal representation. Two separate lines of enquiry were pursued with respect to the activity of cells in the rodent hippocampus. First, what influence does geometry exert on the cells of the hippocampus? The first experiment that addressed this question involved the creation of a novel environment that

allowed the boundaries constituting that environment to be gradually deconstructed. The questions considered were, i) whether or not boundaries/barriers were necessary to maintain the spatial firing fields of hippocampal neurons?, ii) would cells always express fields in close proximity to boundaries?, and iii) could the firing fields of hippocampal neurons be controlled by a single polarising cue without any barriers present? The second experiment extended the findings of the first by examining the effect of extending existing boundaries and observing what changes, if any, occurred to the activity of hippocampal cells. Additional questions about learning-related changes occurring over trials were also considered. In particular, would exposure to an extended environment alter the cell activity seen in the non-extended, bounded environment? Learning-related changes were further examined in the final experiment which focused instead on contextual influences on hippocampal firing. Hippocampal neurons were found to acquire a discrimination between two neighbouring locations over the course of several days and weeks. Following this, the context of the environment was altered to see whether the acquired discrimination was transferred to the novel context. The results of these experiments are incorporated into, and extend, an existing model of neuronal processing in the hippocampus.

It is important to recognise that there are different coordinate systems in which it is possible localise oneself. Fundamentally, there are two different systems around which a coordinate system can be organised; the individual, and the space in which that individual is located. Under such a simple formulation a coordinate space defined with reference to the individual is called an egocentric coordinate frame. Egocentric coordinate systems include all the

receptive surfaces the individual possesses; examples include retinotopic (eye-centred), tonotopic (tone-centred) and somatotopic (body-centred) systems. The other type of coordinate system is an allocentric, or other-centred space (O'Keefe and Nadel, 1978). This is defined with reference to the world "other" than in terms of body-centred coordinates. Unlike egocentric coordinate systems it is much harder to typify what is relevant for locating oneself in an allocentric coordinate system. Despite this, considerable progress has been made in recent years in terms of elucidating the underlying mechanisms. For example, it is known that certain features of an environment are more important than others in determining what behavioural response is appropriate (Deacon et al., 2001;Dusek and Eichenbaum, 1997;Sutherland et al., 1983). Also known is that certain brain areas and networks are responsible for representing non-egocentric space and that other areas underlie different types of navigational strategies (O'Keefe and Nadel, 1978).

The brain structure most strongly linked to the representation of allocentric space is the hippocampus. Residing in the medial temporal lobe, the hippocampus displays remarkably stereotyped patterns of organisation and structure across species. Understandably this has evoked intense curiosity as to what exact function might underlie this structure. Evidence from humans with medial temporal lesions has revealed that this structure has an intimate involvement in memory and learning, a position supported by many animal and modelling studies (Moser and Moser, 1998;O'Reilly and Rudy, 2001;Scoville and Milner, 1957). Pharmacological, molecular genetic and electrophysiological disruptions restricted to the hippocampus result in huge deficits in navigation and spatial behaviour (Castro et al., 1989;Moser and

Moser, 1998;Sutherland et al., 1983). Furthermore, single and multi-cell recording studies show that cells in the rodent hippocampus are strongly correlated with the location of the animal in space (McNaughton et al., 1983;O'Keefe and Conway, 1978;O'Keefe and Dostrovsky, 1971). The aggregated activity of these "place cells" appears to be responsible for encoding the animal's current location. The discovery of these cells prompted much excitement; here was a neural system that appeared capable of supporting an allocentric representation (O'Keefe and Dostrovsky, 1971). A great deal of subsequent work was aimed at understanding the determinants of the firing of these cells. Place cells uniquely represent different locations with different patterns of activity. Therefore a significant question that arises is what aspects of an environment determine the differences in these responses? Put another way, what information do the inputs to place cells carry? By manipulating the external environment and observing the resulting changes (if any) to the place cells it should be possible to determine which sources of information are important.

In recent years two types of information have been suggested to be important in determining place cells responses – geometric and non-geometric (or contextual) sources (Fanselow, 1990;Lever et al., 2002b;Nadel and Willner, 1980;O'Keefe and Burgess, 1996). Geometric cues are an explicitly spatial variable that seem well suited for driving place cell responses. A recent body of experimental and theoretical work suggests that a major function of the hippocampus is to encode geometry (Anderson and Jeffery, 2003;Hayman et al., 2003;Lever et al., 2002b;O'Keefe and Burgess, 1996).

A succinct definition of geometry is given by Gallistel (1990) that highlights the difference between geometric and non-geometric cues:

> A geometric property of a surface, line, or point is a property it possesses by virtue of its position relative to other surfaces, lines, and points within the same space. A nongeometric property is any property that cannot be described by relative position alone. (p.212)

Indeed, the receptive fields of place cells (place fields) appear to be directly driven by the boundaries of an environment (O'Keefe and Burgess, 1996). If place cells can be driven by boundaries in an environment then it is logical to ask if something less than an extended surface can also drive place cell activity. There is evidence to suggest place cells can be driven by an array of objects in an environment (Cressant et al., 1997). If this is the case, then can place fields also be driven by objects in isolation? How the place cell representation responds to such changes in geometry is a question that is addressed here.

It has also been known for some time that the hippocampus is involved in processing contextual information (Kim and Fanselow, 1992;Phillips and LeDoux, 1992). Hippocampal lesions, for example, impair the ability to use context as an appropriate cue to drive the correct behavioural response (Maren and Fanselow, 1997). Concordantly, place cells appear to be driven by changes in context (Anderson and Jeffery, 2003;Hayman et al., 2003). Given the avowedly spatial nature of place cells this is a surprising outcome, if one

considers that context is not strictly a spatial variable. The receptive fields of place cells attest to the avowedly spatial nature of the signal carried in the hippocampus. It will be shown however that this spatial signal can be modulated by changes in context. Geometry, context and the relationship between them is discussed in greater detail in sections 2.4 and 2.5.

1.2. Role of the hippocampus in learning and memory

The role of the hippocampus in learning and memory was first suggested from human lesion studies. In a seminal paper published in 1957 (Scoville and Milner, 1957) Scoville & Milner evaluated data from 10 patients and showed that following bilateral resection of the medial temporal lobes extensive enough to damage significant portions of the hippocampus, patients showed a "clear and persistent disturbance of recent memory" (p. 20). The data from one patient (H.M.) undergoing experimental surgery to cure intractable epilepsy is particularly relevant. As confirmed by recent MRI evidence, nearly all the hippocampal formation was removed during the operation although small parts were still visible (they were atrophic) (Corkin et al., 1997). Hippocampal patients such as H.M present with a distinctive pathology. Extensive tests over many decades have revealed that H.M. has an anterograde memory deficit that presents regardless of the kind of memory test used (e.g. free recall, cued recall, multiple-choice recognition), the modality the material is presented in or the kind of stimulus material (e.g. words, digits, faces, sounds) (Corkin, 1984;Corkin, 2002). Recent or immediate memory (the ability to repeat or recognise recently presented items) is intact, as is remote memory (e.g. memory for childhood events). In addition, memory impairments are also seen when delays are introduced between presentation

and recall of material. The type of memory that is critically affected in these manipulations is declarative memory (Cohen and Squire, 1980) which encompasses both semantic and episodic memory. Semantic memory is memory for factual information (e.g. what is the capital of Brazil?), episodic memory (Tulving, 1972) is memory for a particular spatio-temporal event that comprises a unique personal experience (e.g. walking down a street in Brasília) (Eichenbaum, 2000). In contrast to declarative memory, non-declarative memory is typically expressed through performance rather than recollection (Squire et al., 2004), with different facets of non-declarative memory dependent on different networks of brain areas (such as the amygdala, neostriatum and cerebellum).

Converging evidence of a profound and severe anterograde amnesia following bilateral hippocampal lesions was also found with two other patients (Penfield & Milner 1958) adding weight to the idea that the hippocampus was critically involved in memory. However, extending these findings to animal models proved problematic. Subsequent investigations failed to find deficits in many traditional laboratory tasks that assay learning (such as operant and classical conditioning tasks) (Nadel, 1968). Part of the difficulty here is methodological; the hippocampus is buried deeply in the brain and has many fibres of passage that pass through it. Consequently, aspiration and electrolytic lesions (the type commonly used in lesion studies) can have non-specific, unwanted effects (Jarrard, 1989)[1]. Recently, neurotoxic agents such as ibotenic acid, allied with more accurate stereotaxic methodology have

[1] It is also noted that similar damage to fibres of passage and surrounding brain areas occur during surgical procedures in humans and also make the interpretation of human brain lesion data difficult.

allowed lesions to be targeted to the hippocampus and reasonably "pure" lesions to be made. The advent of gene knockouts, antisense and other molecular biology technologies promise even greater spatial (and temporal) control over lesion sites (Jarrard, 2002).

Further methodological issues include the type of task used to assay the result of the lesion, the type of cues used in that task and the training given to the animal (Alvarado and Rudy, 1995a;Eichenbaum et al., 1988). For example, the radial arm maze task requires an animal to visit each arm of an 8- or 17-armed maze once and only once in order to receive a food reward at the end of each arm (Olton and Samuelson, 1976). Arms are not re-baited and visits to an arm that has already been visited are scored as errors. The radial maze task therefore requires animals to remember where they have been. Normal rats tend to visit each arm once and only once although there is no obvious pattern to their search behaviour. It therefore seems they are able to remember which arms they have visited. If extramaze cues are all that indicate food location (forcing the animal to use a spatial strategy) control rats perform with high levels of correct responses. However, rats with lesions of the fimbria-fornix[2], hippocampus or entorhinal cortex make many errors (Olton et al., 1978). Specifically, they are unable to remember which arms they have already visited but are able to remember which arms were never baited. The patterns of behaviour the animals exhibited following these lesions were effectively random, although some would use specific strategies (successive 90° turns, for example). That rats were unable to remember which arm they had visited suggested a difference between working memory (a list updated on a moment-

[2] The fimbria fornix is a major input and output pathway of the hippocampus connecting it brain regions including the diencephalons, striatum, forebrain and prefrontal cortex.

to-moment basis) and reference memory (longer lasting and spanning across trials and delay periods) (Olton, 1979). Consequently it was proposed that the hippocampus was required for the solution of working memory tasks but not reference memory tasks (Olton, 1979). The arrangement of cues contained in the experimental space can affect the outcome of such tasks. If the cues that specify food reward are local, intramaze cues (i.e. a cued version of the radial maze) then the number of errors can be attenuated (Winocur, 1982). So it appears that the hippocampus is needed for remembering where an animal has been if the cues that indicate location are distant, extramaze ones but not if they are local, intramaze ones.

However, somewhat confusingly, findings from another hippocampal-dependent task that *does* require an intact reference memory show that the hippocampus is also involved in reference memory. The Morris water maze task consists of a circular pool 1-2m in diameter filled with opaque water (Morris, 1981;Morris et al., 1990;Sutherland et al., 1989). A rat is released into the pool and swims around until it encounters a small platform (hidden under the surface of the water) upon which it can stand. This is the hidden version of the water maze; the visible version is where the platform is above the surface of the water from the start of the trial. As with the radial maze, the location of the platform can be signalled by a cue (the cued water maze task), obviating the need for a spatial strategy. Normal rats learn to find the hidden platform rapidly – removing the platform on a probe trial results in the animal spending most of its time in the region previously occupied by the platform. Lesioned animals (fimbria-fornix or hippocampus) cannot locate the platform if it is hidden but can locate it if it is visible (Sutherland et al., 1989). As with

the radial maze this affect can be ameliorated if the task is a cued one.

Despite the failure of lesioned rats on these tasks, pre-training can reduce much of the deficit seen in these animals (Day and Schallert, 1996;Jarrard, 1993;Morris et al., 1990). Using a cued and "place" version of the radial arm maze task, Jarrard (1993) found that rats with lesions of the hippocampus made many errors in the place version, but not the cued version. The impairment seen in the hippocampal group could be attenuated if the animals were trained on the task before the lesion surgery occurred (Jarrard, 1993). This finding was replicated by (Morris et al., 1990). Here rats with lesions of the hippocampus, subiculum or hippocampus and subiculum were assessed in the water maze. Pre-training was sufficient to overcome the effects of hippocampal and subicular lesions alone but not combined lesions. The particular type of training employed also appears important. If lesioned animals are trained first with a very large hidden platform which is gradually shrunk over the course of many trials then they can successfully perform the hidden version of the water maze (Day and Schallert, 1996). These studies show that the mnemonic deficits seen with hippocampal lesions in the radial and water mazes can be attenuated with careful pretraining.

1.2.1. Conclusion

From the preceding it is evident that the hippocampus has a clear involvement with memory. Evidence from human lesion patients' shows the particular type of memory affected is that for events, episodic memory. The results from patients such as H.M. show this type of deficit is global; it doesn't matter which modality is tested, or which test is used to probe it. However, it also

seems that memories acquired before hippocampal lesions can persist and influence behaviour. Together with the temporal gradient seen in hippocampal patients, this raises the possibility that the hippocampus is only temporarily involved in memory, particularly with establishing the initial memory trace (McClelland et al., 1995). Moreover, the pattern of deficits seen in hippocampal animals on different types of tasks suggests the hippocampus may underlie specific types of processing capabilities. The lack of an effect on classical conditioning and operant tasks, and the presence of an effect on spatial tasks, points to a specific involvement of the hippocampus in spatial processing.

1.3. The hippocampus and spatial processing

Prior to work suggesting the hippocampus of animals is involved in spatial processing it had been suggested that rats may use a representation akin to a map to find their way around an environment (Tolman, 1948). This proposal was based on behavioural evidence from rats trained to find a goal via an indirect route. If this route was subsequently blocked off and a direct route was instead made available, then rats chose the direct route more often than if their choices were random. Tolman concluded that despite never having travelled the direct route before, the rats knew the direction in which the goal lay and were therefore able to compute a path to the goal. The representation that allowed such a computation to be performed Tolman called the "cognitive map". It can be concluded from the findings in the radial maze that a rat is able to continuously update the representation of its position in the environment – this is what Olton had referred to as spatial working memory. The hippocampus seems to be heavily involved in this type of memory.

However as discussed above it also seems as though a longer term aspect to memory, spatial reference memory, is also hippocampal dependent, as revealed by the water maze task.

One of the most relevant accounts of hippocampal function in spatial processing is that of O'Keefe & Nadel (1978). Based on an extensive review of the rat learning literature and the discovery of place cells in the hippocampus, they proposed the hippocampus as the site of Tolman's cognitive map; the neural substrate of an animal's internal representation of space. Importantly, they argued that an animal was able to locate itself independently of any single localized view of the environment and was thus freed from the constraints of depending on any one particular landmark or object. The units of this map were proposed to be the hippocampal place cells that O'Keefe & Dostrovsky (1971) had discovered some years previously. As well as laying the foundations for the cognitive map hypothesis of hippocampal function, O'Keefe & Nadel also elucidated other, non-hippocampal based, strategies that animals could use to navigate through an extended space.

Part of the appeal of the cognitive map hypothesis was in the flexibility that such a system possesses. Previous thinking about animals' behaviour was largely limited to simple stimulus-response (S-R) or S-S interactions with the environment (Restle, 1957). The conception presented by O'Keefe & Nadel placed S-R and S-S-R type interactions at the base of a series of possible strategies that could be employed to solve a particular navigational problem. The most basic and least flexible strategy is what they termed a route

navigation strategy consisting of simple chained S-R-S commands. Within such route (or taxon) strategies, the emphasis can be placed on either the stimulus component (called a guidance) or on the response component (an orientation or direction). Guidance's draw attention to a particular stimulus or object and require a certain approach or relationship to be maintained to the stimulus. The behaviour employed to achieve this end is not specified by the guidance. With an orientation/direction the emphasis is shifted to the response required rather than the stimulus itself, although the response often involves maintaining an egocentric relationship with some cue/ object. Some of the advantages of route strategies are that they are rapid and easy to use. However, they are also inflexible, can be rendered redundant by environmental change and must also be used in the correct sequence if a goal is to be successfully reached.

In contrast to taxon strategies, map-based navigation (or *locale* navigation) is highly flexible because of the freedom from specific behaviours and/ or objects. For example, if a particular landmark is used in a route strategy, destruction of this landmark will likely render the route ineffective, however if the same landmark is used on a map, then its destruction can be circumvented by the utilization of an alternative landmark. However, this flexibility is offset by the relative speed with which a goal can be reached using map-based navigation strategies. Because there are so many alternatives available to an organism using a map, selecting which alternative to use necessarily slows down the navigation process. Importantly, the taxon and locale navigation systems were seen by O'Keefe and Nadel as being instantiated by different neural architectures; the locale system was localized to the hippocampus and

the taxon systems were seen as extra-hippocampal. Therefore, animals that have no hippocampus should not be able to use map-based navigation strategies and would be forced to utilize the less-flexible taxon strategies. Whereas the taxon-based systems use an egocentric reference (where the location of stimuli are coded with respect to the organism), the hippocampus allows localization within an allothetic framework independent of the position of the animal. In addition, route based systems are tied to goals, whereas locale systems are not limited to any specific behaviour, goal or task (O'Keefe and Nadel, 1978).

The cognitive mapping hypothesis argues that the role of the hippocampus in rodents is limited to a strictly spatial one. However, during the course of evolution, it is possible that additional functions have been added to the basic spatial module[3] thus allowing the construction of more complex forms of memory such as episodic memory seen in humans (and possibly the "episodic-like" memory seen in other species; see (Clayton and Dickinson, 1998)). As O'Keefe (1999) points out, the construction of an episodic memory system using the spatial mapping system as a basis would require the addition of a fourth temporal dimension to the three spatial ones already present. Other accounts of hippocampal function claim that the spatial processing occurring in the hippocampus is a specific example of a much more general hippocampal involvement in memory (Eichenbaum et al., 1999;Rudy and O'Reilly, 1999;Sutherland et al., 1983), allowing memory for relationships between both spatial and non-spatial stimuli (Cohen and Eichenbaum, 1991) (although see (O'Keefe, 1999) for a defence of the cognitive mapping position). As

[3] Module is meant here in the psychological sense *not* an anatomical one.

discussed below there are also accounts of hippocampal function that seek to define a role for this structure in the processing of context – a role super-ordinate to a strictly spatial one.

The role of the hippocampus in learning and memory suggests that there must be mechanisms for the storage and subsequent retrieval of this information. Much of what we now know about information storage in neural systems is owed to pioneering work carried out in the hippocampus. Much of this work, however, was predated by theoretical considerations that sought to describe how a brain might go about storing information.

1.4. Mechanisms of information storage

One of the most important proposals in the theory of how information is stored neurobiologically comes from the work of Donald Hebb (1949). Hebb is best known for a neurophysiological postulate stemming from his theory of neural perception that the connection between simultaneously coactive cells becomes stronger (for a review of the development of Hebb's ideas, see (Brown and Milner, 2003)). Therefore the activation of a presynaptic neuron increases the probability of a postsynaptic neuron firing. This is input-specific; two coactive neurons have the connection between them strengthened, but other synapses on either neuron (that are not coactive) are not strengthened. Such an associative memory mechanism has since become known as homosynaptic or Hebbian plasticity. This concept was later extended to include the notion that cells that were asynchronously active would undergo a weakening of the connection between them (Stent, 1973). The three canonical properties of homosynaptic plasticity (activity-dependence, associativity and specificity)

form the modern definition of Hebbian plasticity. As Hebb originally proposed, such a system has the potential to account for situations where associations are made between items or events that occur at the same time. This obviously has direct relevance to conditioning experiments that depend upon the temporal contiguity of stimulus and response. In addition to homosynaptic plasticity, a second form of plasticity can occur that is not dependent on coincident activity in the pre- and postsynaptic neurons. Instead, potentiation can occur via the influence of a third modulatory interneuron (Kandel and Tauc, 1965). This form of potentiation can take one of two forms; associative or non-associative. The non-associative form is purely heterosynaptic whereas associative heterosynaptic plasticity combines elements of both homo- and heterosynaptic mechanisms. The potentiation is enhanced in this case when the modulatory interneuron fires coincidentally with the presynaptic cell. Mechanisms such as these will seen to be important when applied to the forthcoming analysis of place cells.

The difficulty with the Hebb rule is that over time every synapse in a network will become stronger and stronger to the point of saturation, thus removing any selectivity. There are various solutions to this problem. For example, Von der Malsburg (1973) proposed that synapses could be normalised by increasing the strengths of synapses correlated with the cell's activity and also decreasing the strengths of uncorrelated synapses (von der Malsburg, 1973). This is a similar rule to the concept proposed by Stent (1973). A further difficulty addressed by von der Malsburg was the possibility that cells with initially random connectivity may (just due to chance) be influenced by the same stimulus. This would result in many cells becoming tuned to respond to

the same stimulus, again destroying the discriminative ability of a network. The idea of lateral inhibition was introduced to address this problem. With lateral inhibition activity in one cell is passed to nearby cells (via the activity of modulatory interneurons) with the result that it reduces the activity in them. This makes neighbouring cells less likely to respond to the same stimulus as the excited cell. There are many potential normalisation functions such as these, some of which have received neurobiological support (see (Turrigiano, 2000;Turrigiano and Nelson, 2000), for a discussion of such mechanisms). A specific learning function, the Bienenstock-Cooper-Munro, or, BCM rule, is also capable of stabilising the overall level of activity and is discussed in greater detail below (Bienenstock et al., 1982).

There are varying degrees of experimental evidence in support of these theoretical propositions. The principles of heterosynaptic modulation were derived from work in an experimental system (*Aplysia depilans*), homosynaptic, Hebbian learning was first described in a mammalian system (in the hippocampus) by Bliss & Lomo (1973) and there has been recent evidence of a biological instantiation of Von der Malsburgs lateral inhibition ideas at the level of the synapse (Fonseca et al., 2004).

Perhaps the leading proposition for how information can be stored neurobiologically comes from work on a phenomenon called long-term potentiation (LTP). In the hippocampus, when the perforant path was repetitively stimulated at high frequency, a long-lasting increase in synaptic transmission was found to occur between the projections of the perforant path and its synaptic target, the granule cells of the dentate gyrus (Bliss and Lomo,

1973). Subsequent work discovered that the form of plasticity occurring was a homosynaptic, associative mechanism as described by Hebb. It is now clear, however, that LTP is not a single phenomenon but rather that there are many different forms and types of LTP (Bailey et al., 2000). Each of these forms has been observed amongst the trisynaptic pathways in the hippocampus. One unifying principle seems to be that each form has an early and a late phase. The early phase is transient, lasting several hours; the long phase can last beyond 24 hours and is dependent on protein synthesis (Bailey et al 2000, Huang et al 1996).

The converse of LTP is a process known as long-term depression (LTD). Where LTP is a strengthening of the connection between two cells, LTD is a weakening of the connection between them. Similar to LTP, there can also be homo- and heterosynaptic mechanisms with LTD. With heterosynaptic LTD only the strengths of inactive synapses are depressed; in homosynaptic LTD active synapses are depressed[4]. Heterosynaptic LTD is most robust in dentate gyrus, whilst homosynaptic LTD is most reliably elicited in CA1 (Bear & Abraham 1996). Clearly, having mechanisms that allow both the up and down regulation of synaptic weights is beneficial – as noted above, runaway potentiation leads to saturation of synapses and a loss of any selectivity; runaway depression would be similarly catastrophic.

The mechanisms of LTP and LTD are the leading candidates for the neurobiological storage of information at the cellular and molecular level. Although initially described in the hippocampus, the same processes have

[4] At least those undergoing low-frequency stimulation (LFS). This can be contrasted to heterosynaptic LTD occurring at inactive synapses during high-frequency stimulation (HFS) (Bear & Abraham, 1996).

been observed in many other brain areas, including the cortex and the cerebellum. The principle of connection of synchronously active neurons, together with disconnection of asynchronously active neurons, is a theme that will be revisited often in the ensuing discussion.

As mentioned above the discovery of LTP occurred in the hippocampus. Furthermore LTP has been discovered amongst each of the main synaptic pathways found in the hippocampus. Moreover, all of the work described in this thesis involved recording the electrophysiological responses of pyramidal cells in the CA1 region of the hippocampus. In a highly reductionist sense by fully specifying the afferent and efferent connections of a place cell and the information carried by them (and how this is transformed en route) it would be possible to predict the effects of changes to the animals environment (internal and external) on the hippocampal place cell system. Therefore it is important to summarise current knowledge about hippocampal anatomy and the salient features of the physiology of the hippocampus. This should aid understanding of the operations and processing performed by the hippocampal place cell system. A germane example of this is the recent discovery of "grid" cells in the medial entorhinal cortex (Fyhn et al., 2004;Hafting et al., 2005;Sargolini et al., 2006). These cells have receptive fields that appear to regularly tile an environment and have generated great interest and excitement. Indeed there have been very recent incorporations of grid cells into models of path integration and its relationship to place cell activity (O'Keefe and Burgess, 2005).

1.5. Anatomy of the hippocampus

1.5.1. Overview

Much of the following description of hippocampal anatomy can be found in Amaral & Witter (1995). The detailed anatomy of the hippocampus has been influential in many computational models of hippocampal function (Marr, 1971;McClelland et al., 1995;Treves and Rolls, 1992). The neuroanatomical detail of the hippocampus informs the structure of such models and has important functional implications. New anatomical techniques have led to an increasingly refined understanding of hippocampal function; models based on a "trisynaptic" circuit and the lamellar hypothesis (Andersen et al., 1969) have been supplanted by more complex theories based on this new insight.

There are two terms used to refer to the hippocampus; the hippocampal formation and the hippocampus proper – the latter is a subset of the former. The hippocampal formation consists of six different regions; the entorhinal cortex, dentate gyrus (DG), cornu ammonis (CA) fields (which in turn is split into 3 sub-regions, CA1-3), presubiculum, parasubiculum and subiculum(Amaral and Witter, 1995). Following the convention adopted by Amaral & Witter (1995) the hippocampus proper will be taken to refer to the CA fields (the hippocampal formation is sometimes taken to also include the DG).

1.5.2. Position of the hippocampal formation and a coordinate system

The location of the hippocampal formation in the rat brain is somewhat difficult to visualise (see Figure 1); consequently, specifying a coordinate

system along which to orient structures becomes a challenge. The gross, overall shape of the hippocampus resembles the appearance of a seahorse; thus the name hippocampus from the Greek *hippo* – horse, and *kampos* - sea. As can be seen from Figure 1 the hippocampus is bilaterally symmetrical – what may not be apparent is that the left and right hippocampi are joined by the hippocampal commissure anteriorly but are separated posteriorly. The longitudinal (antero-posterior) axis of the hippocampal formation is best viewed as an elongated C-shape that starts at the septal nuclei of the basal forebrain and extends posteriorly and ventrally to the temporal lobe. Because of this the long axis of the hippocampal formation is often referred to as the septo-temporal axis and the orthogonal axis as the transverse axis. The terms proximal and distal are used to refer to locations closest to DG and the rhinal sulcus respectively when discussing locations along the transverse axis of the hippocampal formation. Furthermore the terms deep and superficial are used to refer to surfaces closer to the ventricle and hippocampal fissure respectively (see Figure 1). A major anatomical feature that is apparent in Figure 1 is one of the major fibre bundles of the hippocampal formation – the fornix. This stems from a thin sheet of myelinated fibres that cover the deep surfaces of the hippocampal formation and subiculum; this is the alveus – moving from a temporal to septal location this bundle of fibres becomes thicker and collect together to become the fimbria. As these fibres leave the hippocampus and project ventrally into the forebrain they become the columns of the fornix. The fimbria and fornix carry both efferent fibres from the hippocampal formation and afferent fibres to the hippocampal formation from subcortical locations.

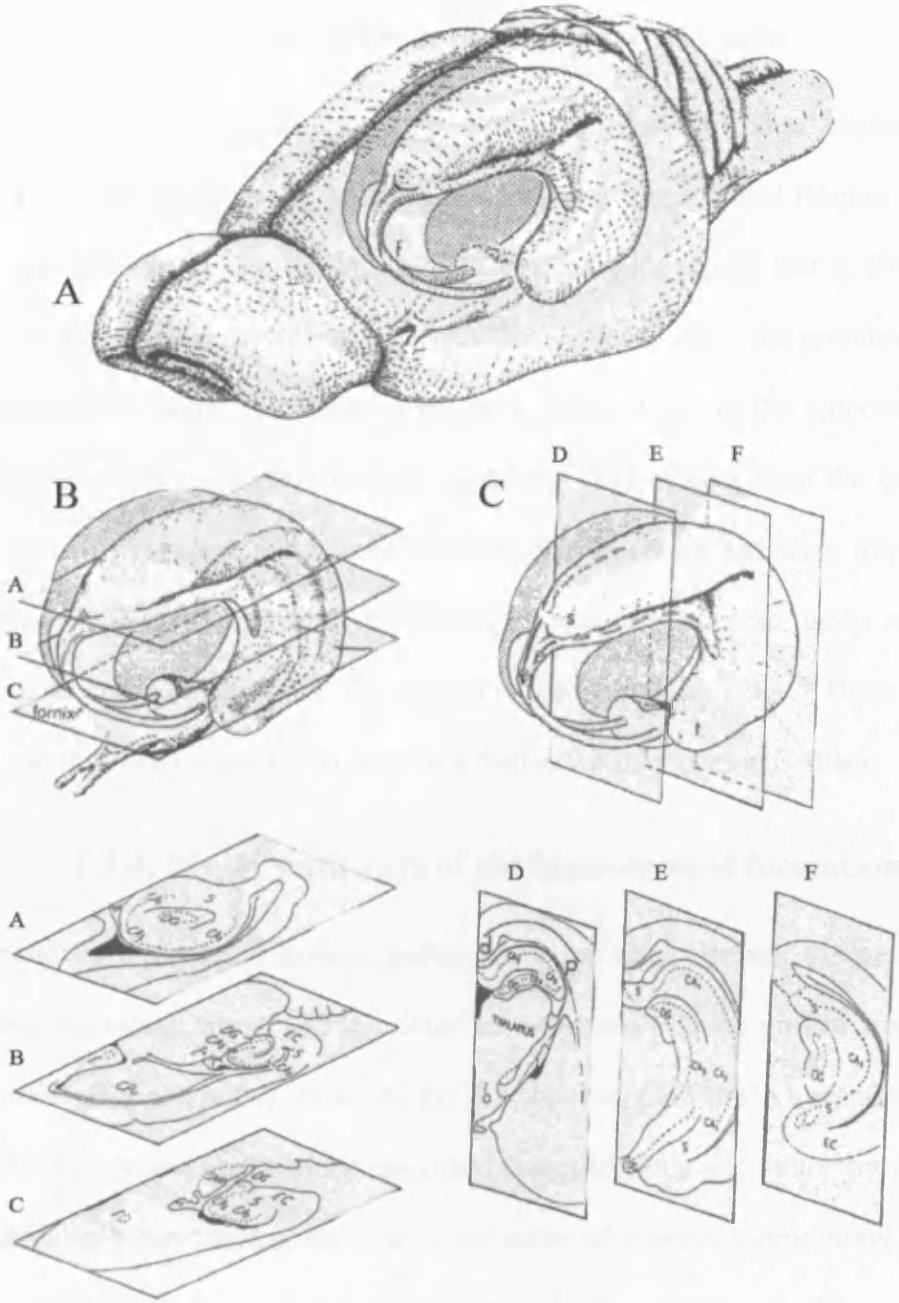


Figure 1. The three-dimensional organisation of the hippocampal formation in the rat. A) The C-shaped hippocampus is shown in relation to the rest of the brain. B) Three horizontal sections at different dorso-ventral levels. The entorhinal cortex is not seen most dorsally (A) but becomes apparent more ventrally (B & C). C) Three coronal sections at different rostro-caudal levels. See text for abbreviations. Taken from Amaral & Witter (1995).

1.5.3. Subfields of the hippocampal formation

As one moves in an anterior to posterior direction and takes consecutive coronal sections different subfields are progressively revealed (Figure 1d-f). At the most septal end the DG and the CA subfields are all that is obvious; moving more posterior reveals the subiculum and eventually the presubiculum and parasubiculum. It is only at the very temporal end of the hippocampus that the entorhinal cortex becomes apparent. It is evident from the coronal sections in Figure 1 that the cell bodies, cell processes and fibre tracts are organised in distinct layers. The principal cells of the dentate gyrus are the granule cells, those of the hippocampus the pyramidal cells. These form layers that wrap around each other in a distinctive interlocking C-shape.

1.5.4. Major pathways of the hippocampal formation

There are three main intrinsic pathways in the hippocampus; the perforant path, the mossy fibres and the Schaffer collaterals. These project from the entorhinal cortex to DG, from DG to CA3 and from CA3 to CA1, respectively. CA1 then projects back to the entorhinal cortex (directly and indirectly via the subiculum) thus closing the loop. This series of synaptic connections forms the traditional hippocampal trisynaptic circuit. Each of the synaptic connections in this pathway is excitatory (using the excitatory neurotransmitter glutamate) and, notably, they are largely unidirectional. This is an unusual situation for cortical areas where reciprocal connections are the rule rather than the exception. The main cortical input to the hippocampus comes from the II and III superficial layers of the entorhinal cortex which in turn receives most of its input from the postrhinal and perirhinal cortices

(echoing the above point about reciprocity of connectivity, the perirhinal to entorhinal connections *are* reciprocally connected). The perforant path input to the DG can be divided into medial and lateral sources which mainly transmit non-olfactory and olfactory inputs respectively. As well as comprising the main input to the DG, perforant path projects to all areas of the hippocampal formation.

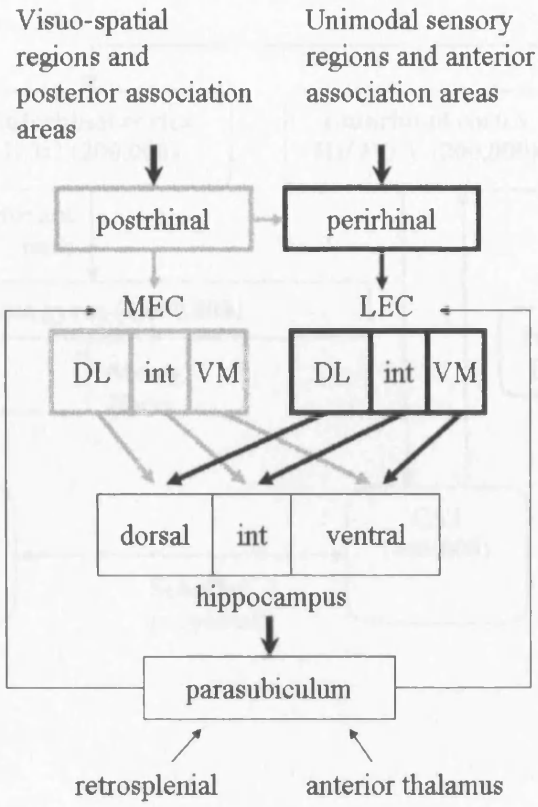


Figure 2. Connectivity between hippocampus and parahippocampal areas. DL, dorsolateral band; int, intermediate band; VM, ventromedial band; LEC, lateral entorhinal cortex; MEC, medial entorhinal cortex.

There is also a dorso-ventral and medial-lateral organisation to the entorhinal to hippocampal projection. The EC is organised into recurrently connected bands that run parallel to the rhinal sulcus and across the medial/ lateral

divisions. These bands project to the hippocampus in a stereotyped fashion; the dorso-lateral part of the EC provides the strongest input to the dorsal aspect of the hippocampus, the ventro-medial projections appear to project solely to the ventral hippocampus (see Figure 2). The dorsal aspect of the hippocampus is more strongly implicated in spatial processing than the ventral aspect (it is also the region where most place cell recordings have been conducted and where all of the recordings conducted here were performed).

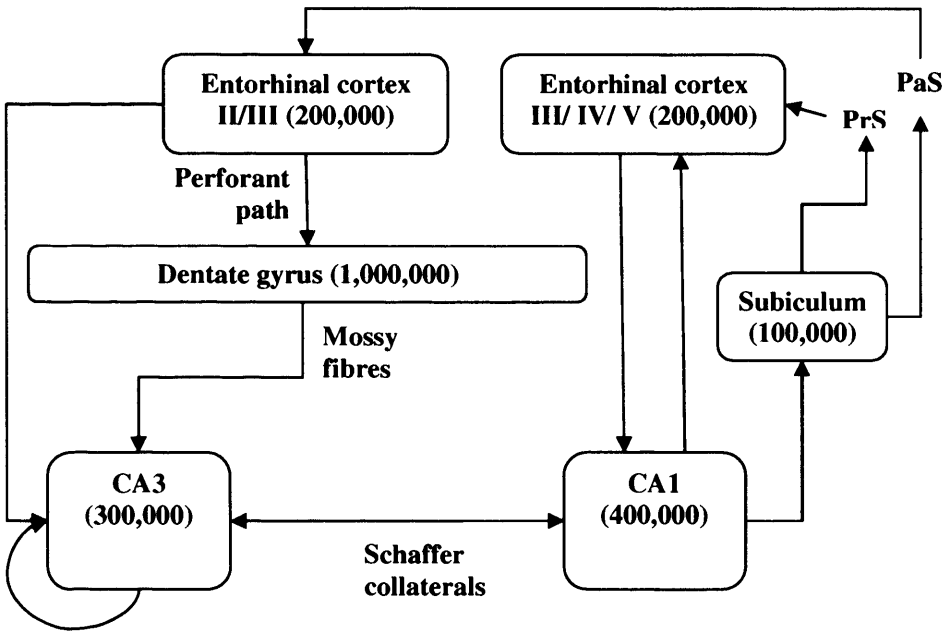


Figure 3. Connectivity of the hippocampal formation. Cell numbers in each region are indicated in brackets. The figures for the entorhinal cortices are the total amount there, not per layer. PrS – presubiculum; PaS – parasubiculum. Adapted from Amaral & Witter (1995).

The unmyelinated mossy fibre projection from dentate granule cells to CA3 is the only extra-dentate projection made from this subfield. There appears to be little topographic organisation to the DG to CA3 projection as all mossy fibres

extend throughout the transverse extent of CA3. It has been estimated that each granule cell contacts and influences only 14-28 pyramidal cells (Amaral and Witter, 1995).

In contrast to the organisation of the DG to CA3 projection, the Schaffer collaterals from CA3 to CA1 are topographically organised. Briefly, CA3 pyramidal cells closer to the DG tend to project to CA1 cells septal to their location. Those located closer to CA1 tend to project to cells located temporally. Additionally CA3 cells located proximal to CA1 have processes that terminate superficially in the stratum radiatum, whereas distal CA3 cells terminate deeper in the stratum radiatum and oriens. So it seems that CA3 cells terminate both on the apical (stratum radiatum) and basal (stratum oriens) dendrites of CA1 cells (see Figure 4 for more detail).

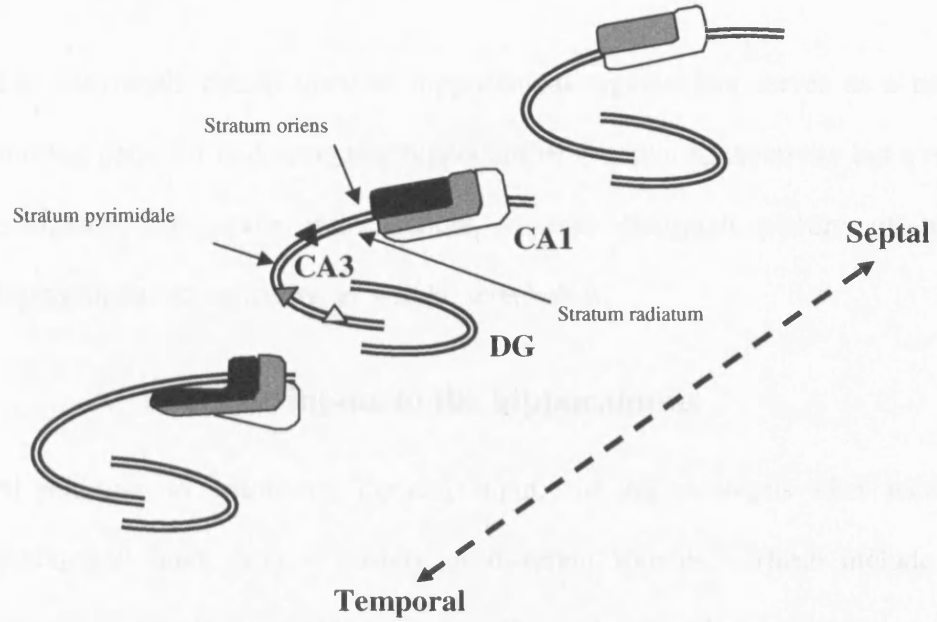


Figure 4. The projections from the CA3 to CA1 fields of the hippocampus. The location of cells of origin is indicated in the middle (coronal) section, and the distribution of fibres and terminals resulting from the cells in the positions in triangles is indicated by the same shading patterns used in the triangles. Adapted from Amaral & Witter (1995).

Although not strictly germane to a consideration of the trisynaptic pathway, CA3 is also massively reciprocally connected. The recurrent connectivity of CA3 has been estimated at between 4-5%; this constitutes the largest single source of input to CA3 (Amaral and Witter, 1995). These connections are also highly organised. Proximal CA3 cells only contact other proximal CA3 cells in the same and adjacent septotemporal levels; projections in the other parts of CA3, however, project throughout the transverse and septotempotal extent of CA3.

The CA1 to entorhinal connection is the first return projection received by the entorhinal cortex from the hippocampal formation. CA1 projects to entorhinal

cortex from the full transverse and septotemporal extent and terminates primarily in layer V (see Figure 3).

The trisynaptic circuit view of hippocampal organisation serves as a useful starting point for understanding hippocampal synaptic connectivity but a more complete view takes into account a more thorough picture of intra-hippocampal connectivity, as will be seen below.

1.5.5. Other inputs to the hippocampus

In addition to entorhinal cortical input, the hippocampus also receives subcortical input from a variety of different sources. These include the amygdala, claustrum, septal nuclei, the supramamillary nucleus, lateral hypothalamus, the anterior and midline portions of the thalamus, the ventral tegmental area, the raphe nuclei, the locus coeruleus and the contralateral hippocampi (Amaral and Witter, 1995). These areas carry diverse types of information relating to arousal, emotional state and autonomic tone and operate via modulatory neurotransmitters such as dopamine (DA), noradrenaline (NA), histamine and serotonin (5-hydroxytryptamine; 5-HT). Additional inputs from the medial septum are important for the generation of the theta rhythm, a prominent rhythmic pattern seen in the hippocampal electroencephalograph (EEG) that has been associated with information processing in the hippocampus. Input from the supramamillary nucleus is also important for theta rhythm generation.

1.5.6. Cell types of the hippocampal formation

The laminar structure of the hippocampus is roughly the same for all fields.

The principal cell type of the hippocampus is the pyramidal cell, the cell bodies of which form the large C of the two interlocking C-shaped bands seen in Figure 1 (parts d-f). This band of cells marks an area of the CA1 field called the stratum pyramidale. The distinctive pyramid shaped cells are oriented so the base of the "pyramid" faces the superficial surface. The dendritic processes of cells in this layer extend from the base both superficially and deep; the basal dendritic tree extends and arborizes in the stratum oriens – a relatively cell free region deep to the stratum pyramidale, deep to which lies the alveus (see Figure 3 for a figure of the projection patterns of different types of cell found in the hippocampus). The apical (superficial) dendritic tree projects in the opposite direction towards the hippocampal fissure. Superficial to the stratum pyramidale in CA1/2 is the stratum radiatum – it is here that the CA3 to CA3 connections and the CA3 to CA1 Schaffer collaterals are located. A difference between CA3 and CA1/2 occurs above the pyramidal cell layer in CA3, which is occupied by the mossy fibre axons that come from the DG – this layer is called the stratum lucidum. Superficial to the stratum radiatum is the most superficial layer of the hippocampus, the stratum lacunosum-moleculare – it is here that the perforant pathway terminates. The somata of pyramidal cells in CA1 are slightly smaller and more densely packed within the stratum pyramidale compared to those in CA2/3. Furthermore, they are reported to have different dendritic lengths depending on the field in which they are located and where in that field they are to be found (Amaral and Witter, 1995). For example, the dendrites of cells in the distal portion of CA3 have total dendritic lengths of approximately 16mm (however, these cells are the largest of the pyramidal

cells in the hippocampus). Conversely the pyramidal cells found in CA1 have smaller total dendritic lengths of roughly 13mm. It should be noted that the stratum oriens contains the majority of the dendritic tree of the pyramidal cells with roughly 20% located in the stratum lacunosum-moleculare.

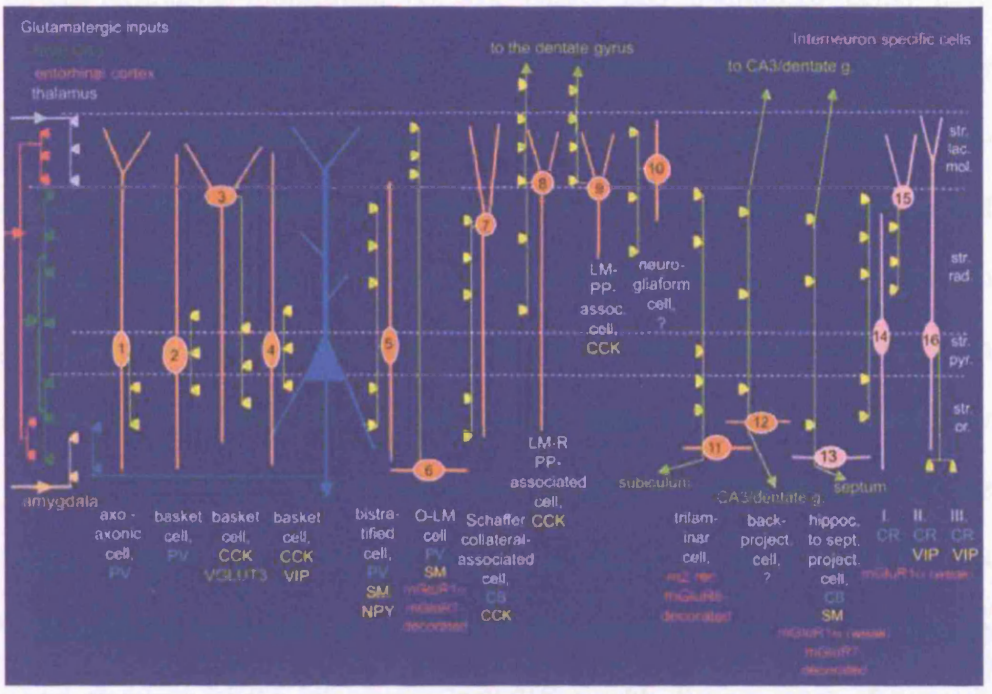


Figure 5. The laminar distribution of dendritic and axonal arbors of different interneuron types in the hippocampus. 12 types of GABAergic interneuron (orange) are shown innervating a CA1 pyramidal cell (blue). 4 types of interneuron (pink) are shown innervating other interneurons. Axons are light green; main termination zones of GABAergic synapses are in yellow symbols. Names of interneurons/ molecular markers are shown underneath cells. CB – calbindin; CR – calretinin; LM-PP – lacunosum-moleculare-perforant path; LM-R-PP - lacunosum-moleculare—radiatum-perforant path; m2 – muscarinic receptor type 2; NPY – neuropeptide tyrosine; PV – parvalbumin; SM – somatostatin; VGLUT3 – vesicular glutamate transporter 3. Taken from Somogyi & Klausberger (2005).

In addition to the pyramidal cell population in the hippocampus, there is a large and heterogeneous population of interneuron's also present. There is an overwhelming array of interneuron subtypes in the hippocampus and

classification schemes have met with varied success (Freund and Buzsaki, 1996;McBain and Fisahn, 2001;Somogyi and Klausberger, 2005). Many of these cells display highly organised patterns of innervation. For example, three different interneuron types with their cell bodies in stratum oriens-alveus (oriens-lacunosum-moleculare (OLM), basket and bistratified cells) have axons that branch and project to different domains. OLM cells project their axons to the distal dendrites of pyramidal cells in the stratum lacunosum-moleculare, basket cells project their axons to the pyramidal cell bodies and their proximal dendrites whereas the bistratified cells project their axons to both proximal and distal dendrites in the stratum oriens and stratum radiatum (see Figure 5). Arguably the projections these cells make must heavily influence the firing properties of pyramidal cells (see Neurophysiology of the hippocampus). -Although functional classification of these cells has traditionally proved difficult it has recently been possible to directly implicate some members of the interneuron population with distinct types of network oscillatory activity seen in the hippocampus (Klausberger et al., 2002;Klausberger et al., 2003)

1.5.7. Inputs and outputs of a CA1 pyramidal cell

Broadly speaking CA1 cells receive inputs from two distinct sources - the Schaffer collaterals of CA3 and the entorhinal layer II and III fibres. However there are some subtle topographical patterns in the afferent connections to CA1. For example, although all portions of CA3 project to CA1, the exact distribution of terminations in CA1 depends on the transverse location of the CA3 cells of origin. For example, proximal CA3 cells (those located closer to DG) tend to project to CA1 septal to their location, whereas distal CA3 cells

(located closer to CA1) tend to project to temporally located CA1 cells. Moreover, each CA3 cell produces a complex branching axonal process that projects to large areas of both the ipsi- and contralateral CA1. Although it was mentioned above that the stratum radiatum is where the Schaffer collaterals are located, the stratum oriens is also highly innervated by CA3 axons. The entorhinal projection to CA1 is the main source of neocortical input to the hippocampus. There are also direct projections from entorhinal cortex to CA3 (see Amaral & Witter, 1995).

CA1 also receives a light septal input (an area implicated in the generation of the theta rhythm seen in the hippocampus, see Buzsaki, 2002 for a review) compared to CA3 – these fibres are concentrated in the stratum oriens. The distal part of CA1 also receives input from the amygdaloid complex (an area strongly tied to fear in classical fear conditioning paradigms, LeDoux, 2003). There are also projections from the thalamus, in particular from the nucleus reuniens, an area thought to be important for multimodal sensory processing.

In terms of the efferent, extra-hippocampal connections of CA1 it appears that there are substantially greater extrinsic connections from CA1 to other brain areas than there are from CA3, leading to a view of CA1 as the output of the hippocampus proper. The outputs from CA1 are organized according to a septotemporal topography. Septal CA1 projects to retrosplenial and perirhinal cortices; midseptotemporal CA1 projects to medial frontal cortex and the temporal levels of CA1 (which also send fibres to medial frontal cortex) projects to the olfactory nucleus and olfactory bulb, the nucleus accumbens, the amygdala and the hypothalamus.

The intra-hippocampal efferents of CA1 project to the subiculum and to the entorhinal cortex. The projection to the subiculum is topographically organised such that CA1 cells in the proximal part of the field project to the distal third of the subiculum and distal CA1 cells project to the proximal portion of the subiculum. CA1 is the first field of the hippocampus to send a projection to the entorhinal cortex – the projections come from all septotemporal levels but terminate most densely in the medial part of the entorhinal cortex, particularly in layer V.

1.5.8. Conclusion

The hippocampus is a highly structured brain area that has attracted considerable interest due to the stereotyped nature of its anatomy. The traditional view of the trisynaptic organisation of the hippocampus has been modified in recent years as it has become apparent that there is greater subtlety in intra- and extra-hippocampal synaptic connectivity than this formulation allows. Although there is some specialization to the type of sensory information that is provided by each of the major cortical input areas to the hippocampus, all sensory areas feed into the hippocampus, which supports the idea that the hippocampus receives multimodal sensory information. An inference that can be made from such anatomical data is that the type of sensory information that the hippocampus is able to respond to is unlikely to be of one particular type; it is more likely there will be a highly abstract relationship between the sensory information reaching the hippocampus and the range of possible responses to this input. The CA1 field is the final "output" stage of the hippocampus. As such the response properties of cells in CA1 should shed light on the nature of the inputs arriving in the hippocampus.

1.6. Neurophysiology of the hippocampus

1.6.1. Rhythmic states in the hippocampus

As alluded to above there are different rhythmic oscillatory states exhibited in the hippocampus. These can be divided into the following categories:

i. Theta rhythm – a population oscillation with large 1-2 mV amplitude with a frequency range of 4-12 Hz. This rhythm has also been called rhythmical slow activity (RSA) (Vanderwolf, 1969).

ii. Gamma oscillations – occur as a result of synchronous synaptic potentials in neuronal groups within a range of 10-40 ms and a frequency range of 20-80 Hz.

iii. Irregular sharp waves – very large amplitude oscillations up to 3 mV, a duration of 40-120 ms and a frequency range of 0.2-5 Hz. Also called large irregular activity (LIA) (Vanderwolf, 1969).

iv. "Ripple" oscillations – very high frequency oscillations of ~200 Hz associated with sharp wave bursts.

Several of these states have specific behavioural correlates. Irregular sharp waves, for example, occur during awake immobility, drinking, eating, face washing, grooming and slow wave sleep and is associated with the synchronous firing of many cells (Freund and Buzsaki, 1996). However the best studied, and most relevant oscillatory state is the theta rhythm. Theta oscillations are most frequent and largest in amplitude in the stratum lacunosum-moleculare of CA1, are consistent along the septotemporal axis of the hippocampus but show amplitude and phase changes as a function of depth (see Figure 6 and Buzsaki, 2002). This depth profile assists judgements of

proximity to the main CA1 pyramidal cell layer when carrying out electrophysiological recording from CA1. The theta rhythm is also present in several other brain areas including the dentate gyrus, CA3, the subicular complex, entorhinal cortex and perirhinal cortex.

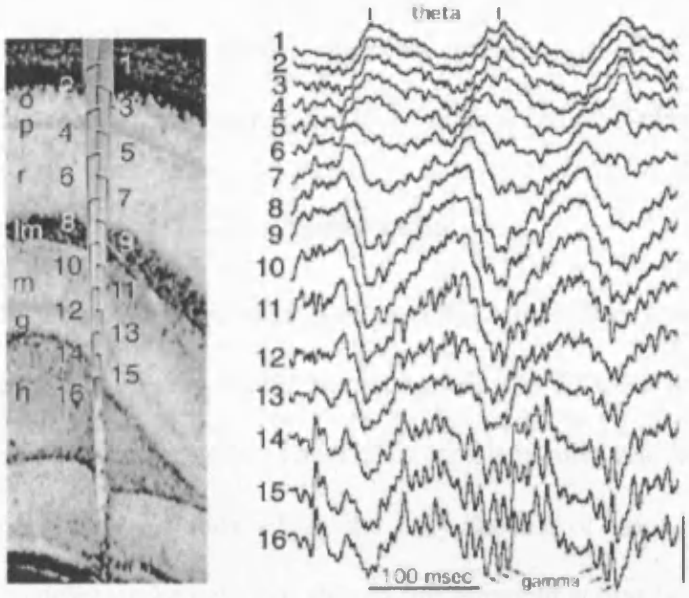


Figure 6. Voltage versus depth profile of theta oscillations in the rat CA1-dentate gyrus axis. Recording sites were separated by 100μm. o – stratum oriens; p – stratum pyramidale; r – stratum radiatum; lm – stratum locunosum-moleculare; g – granule cell layer; h – hilus. Taken from (Bragin et al 1995).

Although no consensus has been arrived at regarding the specific behavioural correlates of theta it has been seen when an animal engages in walking, exploration, sensory scanning, but most reliably during REM sleep (Buzsaki, 2002;Jouvet, 1969). Theta is also strongly linked with long-term plasticity. However, the most relevant correlate of theta for the present analysis is its relationship to pyramidal cell firing.

1.6.2. Cell types in the hippocampal formation

The original characterisations of unit activity in the hippocampus described two main classes of cells; place and displace cells (O'Keefe, 1976;O'Keefe and Conway, 1978;O'Keefe and Dostrovsky, 1971;O'Keefe and Nadel, 1978) that corresponded to complex and theta cells respectively. A place cell is characterised by intense firing whenever the rat's head is in a certain part of the environment dubbed the cell's receptive or "place field". Theta cells are more involved with coding the animals speed (O'Keefe, 1976).

1.6.2.1. Theta cells

When performing in vivo extra-cellular recording from the hippocampus it is usually only practicable to distinguish two different cell types; theta cells and complex-spike cells. Theta cells are distinguishable from complex-spike cells on several grounds. They are only capable of firing single action potentials; complex-spike cells are able to fire bursts of action potentials. Both cell types can display theta phase-locked firing. Theta cells do this wherever the animal is in the environment; complex cells only do so when there is hippocampal theta and the animal is in the place field of the cell (O'Keefe, 1976). Theta cells also have a higher average firing rate and shorter duration action potentials than complex-spike cells and only increase their firing rate if there is a slow wave theta rhythm in the hippocampus (Ranck, 1973). They are particularly related to an animal's locomotion, especially the speed at which it moves (O'Keefe, 1976). These cells have been specifically identified with interneuron's which have traditionally been treated as a somewhat homogeneous group. However recent electrophysiological experiments have begun to elucidate the distinct roles that different types of interneurons may play during different functional states of the hippocampus (Klausberger et al.,

2003).

1.6.2.2. Complex-spike cells/ Place cells

Complex-spike cells are the principal cell type of the hippocampus and correspond to pyramidal cells. Complex-spike cells are capable of firing in bursts where several spikes occur with an inter-spike interval of 1.5-6ms with successive spikes usually displaying decreasing amplitude (they can also fire single action potentials). Compared to theta cells complex spike cells have a low background firing rate, with many of them not active at all (O'Keefe and Nadel, 1978).

As mentioned above a place cell is particularly characterised by firing in restricted portions of space, the cells place field. As the rat's head moves away from the field centre the firing rate of the cell decreases sharply and is almost zero outside the place field. During a typical place cell experiment the position of the animal is recorded with a video camera and action potentials are recorded and stored on computer. Therefore it is possible to represent a place field as a firing rate map and assess what is happening to the cells firing field (see Figure 7). Place field borders can be defined according to criteria that allow the characteristics of a given cell's field to be assessed and make it amenable to statistical analysis. Roughly speaking, typical field size occupies about 13% of the environment (range: 3-50%) (Muller, 1996). The in-field firing rate of cells ranges from 5 to 40 action potentials per second. In a standard bounded, walled environment, firing fields in the centre tend to be circular or elliptical and those near walls tend to have their long axis aligned with the nearest wall (Hartley et al., 2000;Muller et al., 1987;O'Keefe and

Burgess, 1996). Place fields at the edges and corners of environments are generally smaller than those in the central portion (O'Keefe et al., 1998) and fields at the edges of cylindrical environments tend to be crescent shaped (Muller, 1996). Estimates as to the number of place cells that are active (i.e. have fields) in any one environment vary but the active subset is generally less than 50% (Guzowski et al., 1999;Muller, 1996). It appears as though the distribution of place fields over an environment is reasonably uniform although this may be slightly skewed towards having fields next to walls (Hartley et al., 2000;Hetherington and Shapiro, 1997;Muller, 1996).

There are several other physiological properties of PCs that deserve mention here. As an animal moves through a PCs field the cell can fire several bursts of spikes. The frequency between bursts is often in the same range as the EEG theta band (4-12 Hz). As mentioned above, PCs can display theta phase-locked firing when the animal is moving through a cells field. Moreover, it was found that the particular phase of the theta cycle at which a given cell starts to fire is consistent as a rat enters its PF. Furthermore, as the animal moves through the field, the firing precesses on each subsequent theta cycle (O'Keefe and Recce, 1993). This will increase the amount of positional information that is available compared to place cell firing alone; reconstructions of position based on both place cell firing rate and phase information greatly improve reconstruction accuracy compared to rate information alone (Jensen and Lisman, 2000).

1.6.2.3. Other cell types

Cells that display spatially correlated firing are found in several areas of the

hippocampus. Other types of spatial information processed in the hippocampus include signals related to the animals head direction (current and retro-/ prospective), head position (Taube et al., 1990), speed of movement/ location and direction/ place (Cacucci et al., 2004). Granule cells of the dentate gyrus also exhibit spatially related firing patterns (Jung and McNaughton, 1993) as do cells in the subiculum (Sharp and Green, 1994). However, the majority of place cell recording studies take place in either CA1 or CA3 and it is only recently that systematic differences in the firing properties of these two regions have been found (Lee et al., 2004;Leutgeb et al., 2004;Vazdarjanova and Guzowski, 2004).

The entorhinal cortex (EC) is the main afferent structure to the hippocampus and receives multi-sensory input from various areas of association cortex. Electrophysiological recordings from the EC have shown that cells in the dorso-lateral part have sharply tuned place fields with many sub-fields that appear to regularly tile an environment (Fyhn et al., 2004;Hafting et al., 2005;Hargreaves et al., 2005). The information content of the cells in the EC appears to follow a dorso-lateral to ventro-medial gradient such that cells in the ventro-lateral aspect of the EC have the highest information content scores. As such it appears that the input to the hippocampus may be essentially spatial in nature and that the hippocampus is involved in representing spatial and non-spatial signals.

2. The discovery of place cells

Hippocampal anatomy and physiology provide clues as to the information received and transmitted by the hippocampus. Examining the activity of cells contained in the hippocampus of awake behaving animal supplies further information about the functional purpose of this brain structure. All of the work carried out in this thesis involved recording the responses of pyramidal cells in CA1 in response to changes in the sensory environment of awake, behaving animals. These cells' primary correlate is the animals' location in space (see Figure 7 for an example). Therefore it is important to describe the initial discovery of place cells and the subsequent work that has attempted to elucidate what the cells are coding for.

When place cells were first discovered by O'Keefe & colleagues in the 1970's they understandably caused great excitement (O'Keefe, 1976;O'Keefe and Dostrovsky, 1971;O'Keefe and Nadel, 1978). Here were neurons that appeared to participate in the processing of an animal's current location in the external world and as such could be the neural instantiation of Tolman's cognitive map. Since their discovery they have undergone extensive characterisation in many ingenious experiments. The physiological characteristics of place cells are described in detail in the Physiology chapter and so will not be reiterated here. It is relevant instead to discuss some of the salient experimental findings that have elucidated basic properties of the place cell representation.

As far as can be determined it appears as though place cells are active upon initial entry to an environment (Muller, 1996;Muller et al., 1987). Indeed,

their receptive fields, place field maps, can be constructed after several minutes of activity provided the animal sufficiently samples the space bounded by the environment. If the environment is kept constant then a given cell will have the same place field across days, weeks and months (Thompson and Best, 1990). Even if salient spatial cues are removed from an environment then place cell firing can still be reasonably robust (O'Keefe and Speakman, 1987). It seems that there must be a mnemonic component to place cell responding which accounts well with the involvement of the hippocampus in learning and memory. A further conclusion from this manipulation is that a place cell does not fire in response to a single sensory stimulus and must instead be driven over threshold by a collection of cues. However, sensory stimuli can hold potent control of place cell firing (O'Keefe and Speakman, 1987). If the ensemble of cues that specifies a given environment are rotated as a rigid set then the place fields move concomitantly with the cues (Cressant et al., 1997;Muller and Kubie, 1987;O'Keefe and Speakman, 1987). Place cells also continue to show place fields when landmarks are removed from an environment (O'Keefe and Conway, 1978;O'Keefe and Speakman, 1987), a finding examined in greater detail in Experiment 1 and Experiment 2.

Additional support for the idea that there is a mnemonic property to place cells is the finding that even when deprived of all visual cues (by turning the lights off) place fields are still coherent and maintain their original, "lit" firing fields (O'Keefe, 1976;Quirk et al., 1990). Interestingly, rats that have been made blind shortly after birth (and therefore never exposed to any visual stimulus) have place fields very similar to sighted controls although they have

significantly lower firing rates (Save et al., 1998). This suggests that visual experience is not necessary for the formation of place fields although it may be required for the fine tuning of place fields. Indeed place cells appear to be hard-wired, although they can exhibit considerable plasticity (Barry et al., 2006;Bostock et al., 1991;Lever et al., 2002b).

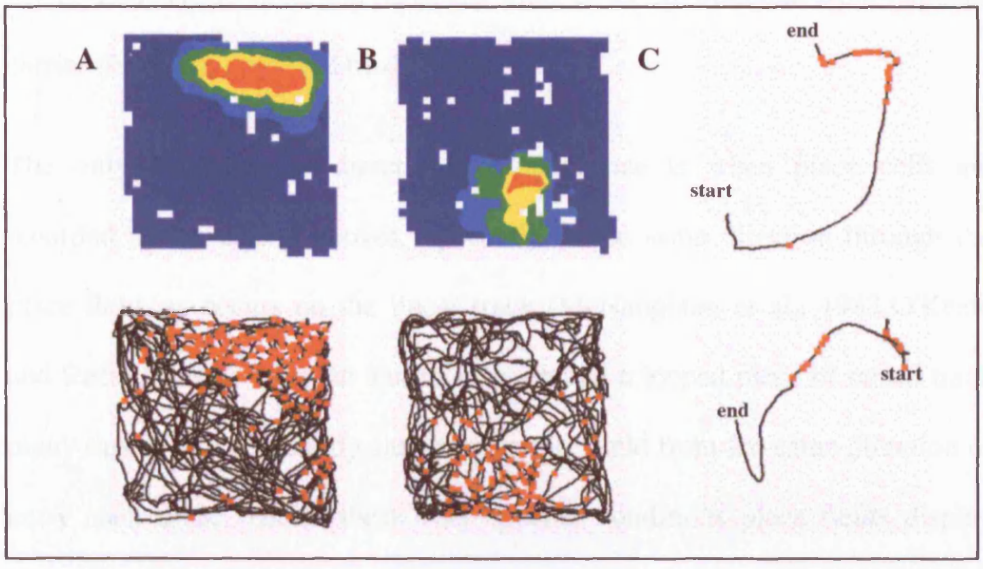


Figure 7. Place fields from the same cell in two different environments. A) Top shows a smoothed contour plot of the cells firing rate. Below shows the raw data collected from the cell. Black lines show the path the rat traversed during the four-minute trial, red squares depict single action potentials. B) The same cell recorded in a different environment (a white box as opposed to the black box in A). Below shows the raw data collected from the cell. Empty (white) areas in to parts of A) and B) are unvisited. C) Independence of place cells on direction of entry into a place field.

2.1. Sensory control of place cell firing

As can be seen from Figure 8 there are multiple types of sensory information that reach the hippocampus by several different routes (Brown and Aggleton, 2001). Perhaps the most intuitive explanation about the location-specific activity of place cells is that the specific conjunction of stimuli that impinge on the rat's sensory apparatus at that particular location cause the cell to reach

threshold and fire (Muller, 1996;Sharp, 1991). Computational models based on such a "local" view hypothesis have been proposed as a means of explaining place cell activity (Sharp, 1991). Such models fail to fully account for much of the experimental data. For example, place cells fire regardless of the direction of entry into the place field (see Figure 7C). Even though a rat has a ~270° visual field, the particular view of an environment from opposite entries to a place field will be different.

The only exception to directional-independence is when place cells are recorded as the animal moves repeatedly in the same direction through the place field, as occurs on the linear track (McNaughton et al., 1983;O'Keefe and Recce, 1993). Here an animal runs around a looped piece of raised track many times, thus repeatedly sampling a place field from the same direction of entry each time. Under these very specific conditions place fields display directional-dependence; if the animal runs in the opposite direction around the track then a different set of place fields are expressed. Additionally, an asymmetric expansion to place fields is seen such that the preferred firing locations shift back towards the direction of travel and the place fields also expand in size (Mehta et al., 1997). Moreover, if salient cues that surround a recording environment are removed then cells still demonstrate the same place fields as before the cues were removed (O'Keefe and Speakman, 1987). This implies that place cells are responding to more than just a particular combination of sensory cues arriving at a certain place. Instead it suggests that place cells are able to maintain location specific firing in the face of environmental degradation and, as mentioned above, that there may be a mnemonic aspect to their firing patterns.

Support for this idea can be seen in experiments when recording in a familiar environment with the lights on can result in one map becoming active, yet during another trial in the same environment with the lights turned off, a different map can become activated (Quirk et al., 1990). Moreover, when the lights are turned on in the dark condition, the dark map can remain active. Additional support is provided by long-term recording studies where the same cells have been followed across days and weeks and seen to maintain the same place fields (Thompson and Best, 1990). The "local" view hypothesis of place cell firing does not therefore explain important experimental data.

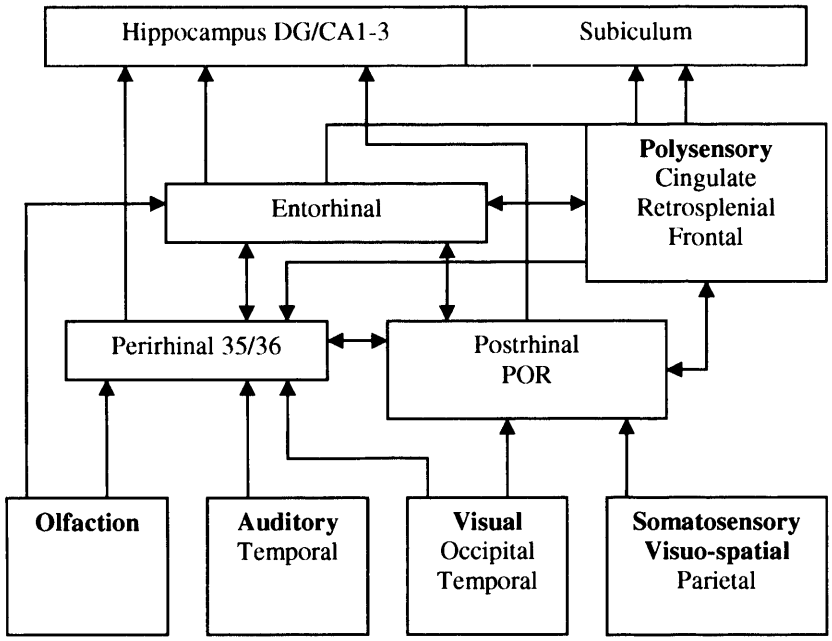


Figure 8. Multiple types and routes by which sensory information reaches the hippocampus. Thickness of the arrows indicates the size of the projection. See also Figure 3 for a closer examination of the connectivity of the hippocampus. Adapted from (Brown & Aggleton 2001).

As demonstrated by cue rotation experiments, sensory information strongly influences the responses of place cells. Place cell firing is influenced and

maintained by many different types of sensory information. The directional orientation of place fields can be controlled by the rats internal sense of direction (Jeffery et al., 1997), with distal cues given primacy for the spatial control of place fields (Jeffery et al., 1997;Jeffery and O'Keefe, 1999). A distinction can be drawn between distal, extramaze cues and local, proximal or intramaze cues. Distal cues can be defined as those that are physically inaccessible to the subject and suffer the little apparent change relative to one another when the subject moves. Local cues can be defined as the reciprocal of this i.e. physically accessible and subject to large apparent change. Thus, it makes intuitive sense that distal cues exert potent control over place cell activity. As mentioned above, a canonical result demonstrating sensory control comes from cue rotation experiments where rotation of a salient, polarising stimulus (such as a cue card attached to an arena wall) results in matched rotation of place fields (Muller and Kubie, 1987). The robustness of place fields in the face of limited cue changes is further illustrated by Muller & Kubie (1987). Enlarging the cue card resulted in no significant changes occurring to place fields, yet removing the cue card resulted in a fields rotating to an unpredictable location. That place fields break symmetry in an otherwise symmetrical environment further suggests the cells are either using another source of information to localise (possibly from the head direction cell system (Taube et al., 1990)) or that there is a mnemonic aspect to place cell activity.

Cue controlled environments have also been important in understanding the determinants of the sensory control of place cells (Shapiro et al., 1997). Here, each arm of a four-arm radial maze contained a combination of local cues

(olfactory, tactile and visual). The maze itself was located within a square-shaped enclosure consisting of floor-to-ceiling black curtains with distal stimuli attached to them. Many different stimulus manipulations were carried out. Following the establishment of place fields in baseline training sessions, double-rotation trials were conducted whereby the distal cues were rotated 90° in one direction and local cues rotated 90° in the opposite direction. Cells were classified according to four categories; i) those that either became silent upon the double rotation or whose fields radically switched preferred firing location, ii) those that appeared to be fixed to the laboratory frame (i.e. maintained their firing fields in the same location upon cue rotation), iii) those that rotated with the distal cues and, iv) cells that rotated with the local cues. The authors also conducted scrambling trials where the relative positions of the local and distal cues were mixed. Now some of the cells had fields that followed particular distal stimuli, others followed particular local stimuli and yet still others were most influenced by a combination of distal and local stimuli. Cells with fields that switched off or radically altered location were argued to be tuned to a combination of local and distal cues. When the relationships between the local and distal cues were disturbed via disjunctive rotations the fields underwent a similar radical restructuring (i.e. they underwent a complete contextual remapping, see below). The most common type of response to double rotation trials was for cells to remap (43%), suggesting to the authors that most of the cells were encoding relationships between the local and distal cues. Interestingly, as exposure to the double rotation trials continued cells stopped following distal cues and increasingly adopted new representations. The manipulations introduced in this study

during a single session were manifold; from a baseline trial to a double rotation to scrambling of distal and local cues (in separate trials). Therefore, in this study, it is possible the observed increase in the proportion of new representations may reflect cue instability rather than a disruption to the relationships between distal and local stimuli. However it is also possible that what was being observed was the gradual development of a new representation of the different environments (see Lever et al., (2002) and below for more details).

The relationship between local and distal cues mentioned above is not quite as straightforward as this simple dichotomy portrays. In the experiments described so far the local cues have essentially been extensions of physical surfaces; they line and help to define those surfaces. The objects that are placed outside the recording arena are, of course, inaccessible to the animal. So both the local and distal cues described so far can be conceived as not constituting real objects that the animal can move around and explore. Such cues should be seen as distinct from local and distal cues and as yet have been investigated in few place cell experiments. Indeed such objects should constitute a separate, third class of cues seen as distinct from both distal *and* local cues.

When a rat does have full physical 360° access to stimuli then objects do not appear to control place fields. This is seen clearly in an experiment by Cressant, Muller & Poucet (1997). Cells were recorded in a circular environment that contained three objects (a wine bottle, a wooden cone and a plastic cylinder). When the objects were located at the perimeter of the

environment and rigidly rotated place fields also rotated. The objects in this instance seem to be functioning as cue cards and distal objects. Yet when the objects were moved to the centre of the arena and rigidly rotated similar rotational control was not found (Cressant et al., 1997). Interestingly a similar lack of local cue control has also been seen with head direction cells in the anterodorsal nucleus of the thalamus (Zugaro et al., 2001) suggesting that such an effect may be a property of the mapping system as a whole. The result seen with place cells is discussed in greater detail in the Introduction to Experiment 1.

2.2. Place cell remapping

Despite the tight control of sensory stimuli over place fields two sufficiently different environments will produce two different place cell representations. Subsequently, the activity of a cell in one environment cannot be predicted from the activity in a different environment (see Figure 7A and B). These different representations are stable over the same time course as described above (weeks to months) and do not appear to interfere with one another (Muller, 1996;Muller and Kubie, 1987). Therefore, place cells are able to represent different environments over extended periods of time. Being able to store different representations of distinct places has obvious advantage for a foraging organism. The reorganization of the place cell representation that is seen in response to an experimental manipulation has been dubbed a "remapping", on the assumption that the representation that is instantiated is both novel and non-overlapping with the previous one (Muller 1996, Muller & Kubie 1987). As remapping consists of a change in the activity of a population of cells, manipulating the circumstances that lead to remapping can

give insight into the mechanisms underlying the formation of the place cell representation as a whole. The phenomenon of remapping has been successfully exploited in studies that have sought to determine the cellular and molecular machinery that may be responsible for place cell activity and place field formation (see for e.g. (Kentros et al., 1998)).

The exact circumstances that elicit a remapping are many and varied. As yet no theory has been advanced that predicts whether a given change to an environment (be it internal or external to the rat) will result in a remapping (Muller and Kubie, 1987). There are indications from the first published reports on place cells that remapping was being observed there (O'Keefe, 1976;O'Keefe and Conway, 1978;O'Keefe and Dostrovsky, 1971). Sometimes it was found that cue manipulations would result in cells switching off or shifting their preferred firing locations to novel parts of the environment. The term "remapping" was used to describe this process by Muller & Kubie (1987). Doubling the size of a recording environment resulted in some place fields displaying a similar scaling. However some cells had fields that behaved as in the earlier experiments, switching off or shifting their fields to novel locations.

A study that provided the first real insight into remapping comes from Bostock, Muller & Kubie (1991). Animals were initially exposed to a white cue card attached to the wall of a circular grey coloured environment. Following training in this environment, the colour of the cue card was switched to white and recording continued. Place cells remapped this new environment by switching off or shifting their preferred firing location. Once

a cell had remapped, all subsequently recorded cells from that animal also displayed altered place fields in the presence of the altered cue card. This implied that the phenomenon was an all-or-none event that occurred within a time frame of 2½ - 3 minutes. This suggested that once place cells had remapped an environment this new representation would persist. Furthermore such a result implied that different hippocampal representations were independent of one another. Since then several other types of remapping have been described. These can be arbitrarily grouped as follows[5]:

i. Rotational (Cressant et al., 2002;Muller and Kubie, 1987)

ii. Geometric (Lever et al., 2002b;O'Keefe and Burgess, 1996)

iii. Contextual (Anderson and Jeffery, 2003;Hayman et al., 2003)

Geometric and contextual remapping can be expressed either partially or completely. Complete remapping is the type that was described in the Bostock et al., study – all the place fields behave coherently and remap as one. Rotational remapping always appears to be complete, except under circumstances where there are disjoint rotations between distal and proximal cues (Cressant et al., 2002;Shapiro et al., 1997). It is possible to have partial geometric remapping (see Experiment 1), partial contextual remapping (Anderson and Jeffery, 2003) and complete contextual remapping (Bostock et al., 1991;Hayman et al., 2003) for example. This is a point that will be examined in detail later.

2.2.1. Rotational remapping

[5] Such a grouping of types of remapping is necessarily based on the conditions that elicit or trigger such reorganisations.

Rotational remapping (or null remapping; (Muller, 1996)) is when the angles and distances between place fields are maintained and the representation rotates concordantly with salient cues. The most straightforward demonstration of rotational remapping occurs when rotation of a salient stimulus results in a matched rotation of place fields. If distal cues are rotated as a rigid set then place fields exhibit a concomitant rotation (O'Keefe and Speakman, 1987). Control over the angular position of place cell firing is not just limited to visual stimuli. Auditory and somatosensory stimuli (O'Keefe and Conway, 1978) as well as olfactory cues (O'Keefe and Speakman, 1987) can also control the angular firing position of place cells. Interestingly, it is possible to decouple place cells from salient stimuli and force place cells to rotate with the rat's internal sense of direction (Jeffery and O'Keefe, 1999). Here place cells were recorded in a cue-controlled environment within which was located a square box that sat on a rotating turntable. A large cue card was attached to the curtain walls that broke the symmetry of the environment. By rotating the box (plus rat) and the cue card different amounts it was possible to see if place fields would follow the card or the rats internal sense of direction. For rats that had never seen the cue card move place fields always followed the card. However rats that had seen the card move had place fields that initially followed the card but eventually rotated with the rat instead. This implies that for animals that had experienced the cue card as unstable the place cells were able to learn that the card was an unstable directional indicator. These animals had learned not to rely on the unstable cue card but instead orient themselves, and their place cells, based on their internal sense of direction. As well as demonstrating another instance of rotational remapping

this study also illustrates that the hippocampus receives multisensory input and that place cells are capable of exploiting these different sources of information.

Although described as remapping, with rotational remapping the same representation is present before and after the manipulation is performed and essentially the same map is present despite being rotated. It seems a different process is occurring with the Jeffery & O'Keefe result as there is a learnt component to the remapping, i.e. cells are learning to ignore the unstable cue and rely on a more consistent source of information. Although the remapping is not learnt, that the process took place over several trials suggests the cells "learn" to not rely on the cue card and rely instead on the rats internal sense of direction.

2.2.2. Geometric remapping

Geometric remapping has also been described (Anderson et al., 2003;Muller and Kubie, 1987;O'Keefe and Burgess, 1996). Most simply, this occurs when the walls of an environment are stretched or gradually deformed and place fields exhibit similar stretching or elongation. This suggests that place cells are responding to the boundaries of the environment, and has led to a model of place field formation whereby place fields are formed by the summation of Gaussian tuning curves oriented perpendicular to the environment walls (O'Keefe and Burgess, 1996). A descendant of this model, the boundary vector cell (BVC) model, is discussed in more depth later. Muller & Kubie (1987) further investigated the effect of boundaries on place fields by recording a standard session, finding place fields, and in a subsequent session,

introducing a barrier into the environment such that it bisected a place field. The effect of such a manipulation was to turn off place fields, an effect which was limited to the barrier-insertion trials as the fields returned in barrier-free trials that followed. Cells further from the barrier remained unaffected. The argument made here was that a subset of cells within a coherent place representation can adjust their activity to accommodate the introduction of a new object.

The effects of inserting a barrier into an environment have also been examined recently with (Barry et al., 2006). Cells were recorded in a square environment and, in a subsequent trial, a north-south barrier was introduced which ran from the north wall approximately ¾ the length of the box. Barrier insertion produced a field-doubling in 3/10 cells, 3/10 either became active or moved adjacent to one side of the barrier, 2/10 were unaffected, one turned off and the final cell moved away from the barrier. Field doubling here means that if a cell expressed a place field to the East of the North wall then, following barrier insertion, the cell would express a field to the East of the inserted barrier *whilst maintaining* its field to the East of the North wall. These responses are more varied than those described by Muller & Kubie (1987). It appears here that barriers are having an effect beyond simply turning a cell off. The findings from Barry et al., are well accounted for by the BVC model (see below).

This notion of local remapping (Knierim, 2003) has recently been extended with the suggestion that there are two different functional classes of place cells (Rivard et al., 2004). The first type was traditional place cells. The second

are what the authors referred to as "object cells". This type of cell is claimed to signal proximity to a barrier introduced into an environment. When the barrier was moved the cells shifted their fields to follow the barrier; when it was removed the cells stopped firing. Interestingly when the barrier was moved to a novel environment (which itself induced remapping) the barrier cells continued to fire at the barrier.

These findings suggest that place cells have a special relationship with barriers. Responses such as those seen in the Rivard et al., experiment were not seen in the Cressant et al study where superficially similar manipulations were carried out – i.e. objects were moved around the environment. Yet the place fields in this experiment failed to follow the objects when they were moved. This may be due to the extended nature of the barriers used in Rivard et al., and the more localised, point-like nature of the objects with Cressant et al.

The type of local remapping seen in the Rivard et al experiment can be contrasted to a more gradual type of remapping that takes place over days and weeks (Lever et al., 2002b). This involves a gradual, graded change to the whole representation that occurs with the continued experience of a place. It should be noted that this type of remapping may be under-reported in the literature. In order to see this type of remapping it is necessary to record place cell responses from the first exposure of a rat to the environment through to the termination of the experiment. This is not always carried out. Frequently rats are thoroughly trained to forage for food reward before recording begins so that when recording starts there will be good spatial sampling of the

environment. The unfortunate result of this is that an important and interesting period of the rats' experience of an environment is ignored. It is only when the representation is well established and any incidental learning has occurred that place cell responses are collected. Partial remapping in the sense of initially similar but gradually diverging place cell representations has been seen most reliably in studies that have exposed animals to two different but highly similar environments whilst capturing place cell activity from the initial exposure. In particular, Lever et al., (2002) found that cells repeatedly exposed to two differently shaped environments had initially similar representations that gradually diverged. This difference was maintained over a month and transferred across new enclosures of the same shape. Such gradual geometric remapping suggests that features of the environment can be learnt and that this learning can be manifested in the activity of place cells.

2.2.3. Contextual and partial remapping

For the sake of consistency and simplicity, contextual remapping will be referred to as what happens when cells switch their fields on or off or shift them to unpredictable locations. The circumstances that elicit such a remapping are numerous and varied. These include changing the colour of the environment (Anderson and Jeffery, 2003), the task the rat is performing (Markus et al., 1995), even changing the expectations or intentions of the rat (Wood et al., 2000) can cause such a restructuring. This type of remapping has been previously referred to as complex (Bostock et al., 1991). In the studies just mentioned a different representation is instantiated for the same space i.e. a non-spatial change has occurred that has caused a remapping. Such evidence takes us away from the original conception of the hippocampus

as something that underlies purely spatial computations. These changes are obviously different to the geometric type of remapping referred to above. Relating findings from context conditioning and place cell remapping, Moita et al., (2003) found partial contextual remapping in rats that had undergone contextual fear conditioning. This effect was specific to the training context (where the shock was experienced) and did not transfer to a control context. The remapping was partial; for example, one cell of a simultaneously recorded pair exhibited the same field before and after conditioning, whereas the other remapped by shifting its preferred firing location (Moita et al., 2003). This finding echoes the first report of partial remapping following a non-spatial (contextual) change to the environment (Anderson and Jeffery, 2003) and suggests that remapping is not an all-or-none event, contrary to the predictions generated from some modelling efforts (Kali and Dayan, 2000;Samsonovich and McNaughton, 1997) and the Bostock et al., finding described above. This also stands in contrast to earlier findings of partial remapping to explicitly spatial changes in the environment (Anderson and Jeffery, 2003;O'Keefe and Burgess, 1996). In the first demonstration of partial contextual remapping, it was found that none of the simultaneously recorded sets of cells responded in a homogeneous fashion (Anderson and Jeffery, 2003). Instead, cells were influenced by different subsets of stimuli, leading the authors to propose a model whereby the contextual cues selectively activate the spatial inputs to a place cell. This model, and a subsequent extension, is elaborated in greater detail below. It seeks to explain how inputs to place cells are integrated to form coherent place fields in a variety of different circumstances. The model serves as a useful framework in which to organise thinking about how inputs

to place cells are arranged and how they relate to one another. Specifically, it details how geometric and non-geometric inputs are organised and how they influence place fields. As the work carried out for this thesis involved the manipulation of geometric and contextual cues it serves as the most relevant account of hippocampal processing. However this must be placed in the context of other important models of hippocampal place fields.

2.3. Models of hippocampal place fields

It is pertinent at this point to move to a broader discussion of computational models that seek to explain the phenomenon of place cells, including the formation and remapping of place fields. Different models of the hippocampus attempt to account for different aspects of its functioning; from the storage and recall of memory sequences (Lisman and Otmakhova, 2001), the theta phase precession of CA1 cells (Bose et al., 2000), to those solely concerned with place field formation (Hartley et al., 2000). Each of these models are based around the neuroanatomy of the hippocampus to varying degrees, with some focusing strongly on the recurrent collaterals seen in CA3 (Samsonovich and McNaughton, 1997) with others taking only superficial anatomical detail into account (Yoshida et al., 2002).

All models of place cell activity have a point at which sensory input enters the network and eventually arrives at the place cell layer. As described above, place cells do not appear to be driven directly (in a one-to-one manner) by sensory stimuli. Rather, there is a high degree of pre-processing that has occurred to incoming sensory information such that it is transformed in some manner (for example by the addition of a head-direction signal). The exact

nature of the sensory input in the different models varies. These include a snapshot of the cues immediately viewable by the animal at each location (Sharp, 1991), isolated points on a cylinder wall (Touretzky et al., 2005) and entire walls of an environment (Hartley et al., 2000;Kali and Dayan, 2000). These inputs can then be passed on to another stage where the input patterns undergo an orthogonalisation process or can be passed straight on to the place cells themselves.

As mentioned above, one of the dominant themes in several models of hippocampal function is the role of the recurrent connectivity seen in CA3. There are two main ways in which this has been exploited; as a neural instantiation of a pattern completer viewed as being important for accurate memory recall (Hasselmo et al., 1996;Marr, 1971;Rolls, 1996) or as an attractor network capable of explaining various facets of the place cell phenomenon (Kali and Dayan, 2000;Samsonovich and McNaughton, 1997). Pattern completion is a mechanism that allows the presentation of a partial set of cues to trigger the retrieval of the full set, and for a complete, entire memory to be retrieved. The high degree of interconnectivity in CA3 has led to the idea that, coupled with Hebbian synaptic plasticity (see Mechanisms of Information Storage above), this area could support pattern completion.

Another attractive anatomical feature of the hippocampus is the organisation and position of the dentate gyrus. Because the dentate contains an order of magnitude more neurons that its principal afferent (entorhinal cortex) and efferent (CA3) structures it is possible that pattern separation could be supported here. Pattern separation is a mechanism whereby highly similar

input patterns are made more dissimilar (orthogonalisation) (Marr, 1971;McClelland et al., 1995;Treves and Rolls, 1992). Therefore, it is possible that dentate gyrus can store very sparse, intermediate representations based on information transferred from entorhinal cortex and pass these non-overlapping inputs to CA3. Pattern separation is an important intermediary step in the storage of information in the hippocampus. If two representations stored in a network of units happen to share a high number of the units then the representations can be said to be overlapping. Conversely, if the representations share a small number of units then they can be said to be non-overlapping. Overlapping representations suffer from the problem of interference. Following reactivation there is the potential for the wrong representation to be retrieved because they share so many units. Because of this it helps if highly similar patterns of incoming information are made more dissimilar i.e. separated.

Recent evidence from two different groups has emphasised the distinct roles played by CA1 and CA3 in pattern separation and pattern completion (Lee et al., 2004;Vazdarjanova and Guzowski, 2004). In the first experiment rats were trained to run on a circular track with local and distal cues in a standard relationship to each other (Lee et al., 2004). The two cue sets were then rotated either 45°, 90°, 135° or 180° relative to each other. In the 45° rotation condition both CA1 and CA3 maintained highly similar firing profiles to the standard, un-rotated condition. With rotations greater than 45° the representation in CA1 lost coherence (i.e. remapped). In CA3 however there was a much higher degree of concordance between the representations in standard condition and the rotated conditions, with CA3 place fields rotating

with the local cues on larger mismatches. Therefore it appears that, under these conditions, CA3 is performing a pattern completion process and CA1 a pattern separation one as suggested by both anatomical and modelling data.

Similar results were seen in a second experiment that used a different methodology to examine cell activity (Vazdarjanova and Guzowski, 2004). By examining the products of two different genes (*Arc* and *Homer1a*) it is possible to infer the activity history of individual neurons (Vazdarjanova et al., 2002). Following the activation of a given neuron *Arc* is transcribed first followed by *Homer1a*. It is therefore possible to reconstruct the activity history of large ensembles of neurons by looking at the distribution of *Arc* and *Homer1a* gene products. Neurons containing the *Arc* gene product are activated 2-15 minutes prior to sacrifice and those containing *Homer1a* transcripts are activated 25-40 minutes earlier. Using this technique it was possible to observe changes in CA3 and CA1 in response to alterations to the recording environment. Following small changes to the recording environment there were a greater number of neurons in CA3 than CA1 with overlapping profiles of *Arc* and *Homer1a* staining. This suggests that the modified environment is treated as more similar in CA3 than in CA1 (i.e. pattern completion is occurring in CA3 and separation in CA1 as with Lee et al). With large changes to the environment (where the animal was introduced to a new context) however, this pattern reversed, with CA1 showing more overlapping staining than CA3. This suggests that the representation of this new context was more orthogonal in CA3 than CA1.

The role of CA3 as an attractor network has been explored by several

computational models of hippocampal place cells (Brunel and Trullier, 1998;Kali and Dayan, 2000;Samsonovich and McNaughton, 1997) and the head direction system (Redish et al., 1996). An attractor can be visualised as a two dimensional surface on which is located each place cell. Each place cell is located at the centre of its peak firing and place cells effectively tile a two dimensional surface. The closer a cell is to its neighbour the stronger the connection that exists between them and the further away cells the weaker the connection. If a network following these principles is initialised with random activity levels it will eventually settle into a stable state such that an activity bump is seen at a random location. This activity bump can be thought of as corresponding to the overall population activity seen in the hippocampus – the peak of the bump corresponds to the animal's current location; as it moves through the environment the bump moves to reflect this.

The original application of an attractor network to model elements of the place cell phenomenon consisted of a continuous set of attractors instantiated in CA3 that made several relevant experimental predictions (Samsonovich and McNaughton, 1997). The first of these was that given two highly similar inputs the attractor network would produce the same output; because the input fell within the set that defines the attractor the attractor would therefore resolve the inputs to be the same. The result of this is that the place cell activations seen in CA3 are the same. Two associated predictions were also made. The first is the reciprocal of the one just described; that given sufficiently different inputs the place cell activations would be completely different i.e. the system would exhibit a complete remapping. The second prediction was that partial remapping would not be seen. Whilst the model

successfully accounted for some basic experimental findings such as the deformation of place fields in distorted environments (Gothard et al., 1996;O'Keefe and Burgess, 1996) and the directionality acquired by place cells in linear track tasks (Mehta et al., 1997), subsequent experimental findings have undermined some of these predictions. Animals exposed to two highly similar but distinct environments had neither completely unique nor completely identical place cell representations of the two environments (Skaggs and McNaughton, 1998). Additionally, animals exposed to two differently shaped environments displayed highly similar initial place cell activations which incrementally diverged over the course of several days and weeks (Lever et al., 2002b). These findings are counter to the attractor hypothesis described above because it predicts there should be an all-or-none type remapping occurring in these two experiments. Both of these findings are discussed in greater detail below (see the Introduction to Experiment 2 and the Introduction to Experiment 3).

Of particular relevance to the experimental work described later is the Boundary Vector Cell (BVC) model. Although not as contingent on the anatomy of the hippocampus as some of the models described above, it is nonetheless able to account for many significant experimental findings.

2.4. Geometry

The influence of extended surfaces such as boundaries or walls has also been examined with a view to understanding their influence of place cell activity. The most immediate effect of a wall is to constrain the paths that can be traversed through space. Whilst discrete physical objects also possess a

similar attribute (although to a lesser degree) they are more useful as simple landmarks enabling accurate navigation.

During a typical place cell experiment an environment is usually defined by its immediate walls that completely enclose or define a space. Even in the case of arenas without walls, such as the radial maze, the space through which the animal moves is restricted (in this case because it is elevated above floor level). The main advantages of constraining the area an animal can move around in are obvious: tight experimental control is possible over the area the animal samples and the cues it is exposed to. Additionally, the discharges of a place cell on individual passes through its place field are highly variable (Fenton and Muller, 1998). In order to account for this variability many individual samples of a place field are necessary. By limiting the area an animal can move through, and provided the trial time is long enough, it should be possible to have the animal sample all parts of an environment reasonably equally[6].

Given the above considerations it is desirable to examine exactly what influence boundaries have on place cell activity. A study by O'Keefe & Burgess (1996) examined this by extending the dimensions of a box environment; other experiments have investigated the influence of boundaries by introducing new ones into an environment that previously consisted only of those that made up the arena itself (Muller and Kubie, 1987;Rivard et al., 2004). O'Keefe & Burgess observed that the location of peak firing of place

[6] Initially, rats tend to spend more time at the edges of an environment than in the centre (such "wall-hugging" behaviour is called thigmotaxis). Increasing familiarity with an environment usually results in more time spent away from the edges and a higher number of traversals across the central region.

cells typically maintained a fixed distance to the nearest walls. When the environment was extended, some of the fields stretched along the dimension that was being extended. Similar changes have also been seen across environments of different shapes (Lever et al., 1999). The explanation proposed for these responses is that place cells receive inputs that are tuned to respond to the presence of a barrier at a given distance along an allocentric direction with closer distances leading to sharper tuning (O'Keefe and Burgess, 1996).

The model proposed by O'Keefe & Burgess was later extended and formalised as the Boundary Vector Cell (BVC) model (Hartley et al 2000). The BVC model is a feed-forward model that transforms input from cells that respond to the presence of extended boundaries into the observed place cell responses. Whereas many of these models use the inputs from a collection of point-like stimuli (see (Sharp, 1991), and (Touretzky et al., 2005) for a recent example), the BVC model sees place cell firing as a continuous function of the relative location of environmental barriers. In the model, a given place cell receives feed-forward input from a number of boundary vector cells, each of which fires in the presence of extended surfaces (see Figure 9). A given boundary vector cell fires maximally at a preferred (allocentric) direction and distance from a barrier. The heading of the animal is not important for firing of BVCs and the direction tuning for all BVCs occurs within the same (allocentric) reference frame. Additionally, the sharper the tuning of cells with short preferred directions implies that boundaries nearer to the peak firing will tend to have more influence than those further away.

Simulations using the BVC model have been able to reproduce the results obtained in the O'Keefe & Burgess study (O'Keefe and Burgess, 1996). It is also able to predict what will happen to a cell when the animal is introduced into a new environment providing the likely set of BVCs has been determined from exposure to a previous, unrelated environment. If it is assumed that the reference frame to the place cells is provided by the head direction system, then the BVC model is also able to account for field rotation, as the preferred allocentric tuning of BVCs are determined relative to the same reference frame. A modification of the original model incorporates two additional assumptions (Hartley and Burgess, 2001). First, the influence of a distal visual cue on the directional reference system is proportional to its distance from the rat. This proposal is consistent with various implementations of models of head direction selective cells (Skaggs et al., 1995;Zhang, 1996). Second, and most importantly for the experimental work described later, is that BVCs can become modulated by colour. This results from the continued presence of colour variation along a boundary to which a BVC responds. The authors point out that the second assumption implies the presence of synaptic learning that occurs outside of the hippocampus. Some of the work presented in this thesis speaks to this issue via the addition of layer of units that control which sets of BVCs are activated following both learning and colour (or, more accurately, context) changes to an environment.

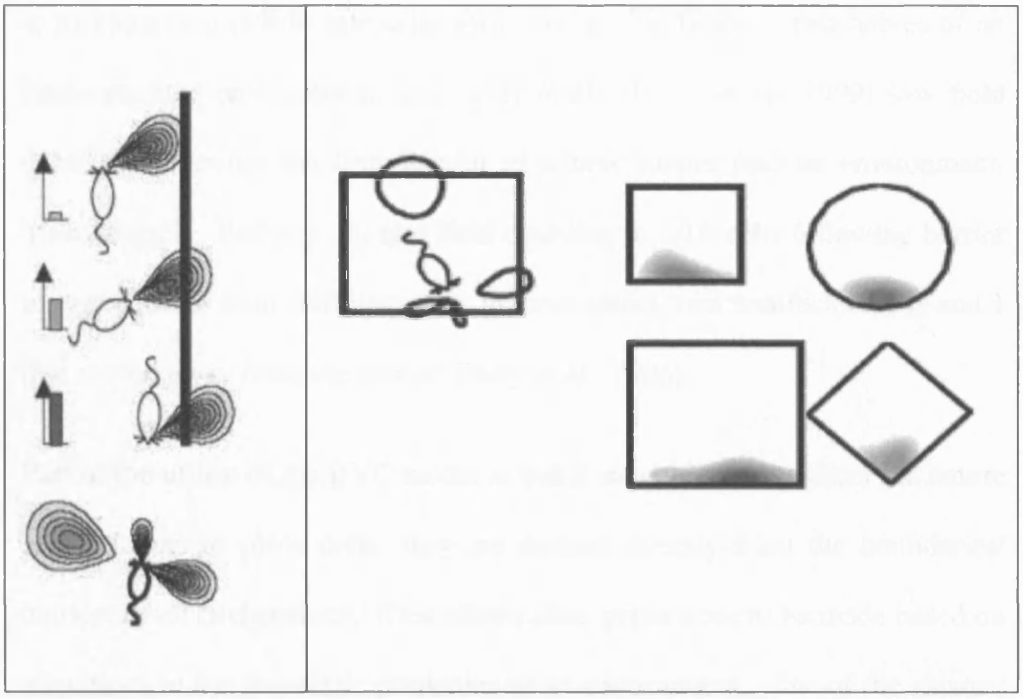


Figure 9. The Boundary Vector Cell (BVC) model. Left: A BVC responds at a preferred direction and distance from a barrier/ boundary. Bar graphs on left of figure depict firing rate of cell. Firing rate increases as distances and directions come closer to the preferred values. The tuning is sharper for shorter distances. Right: Simulations derived from the BVC model based on the 4 inputs (BVCs shown on the left). Taken from Hartley et al., (2000).

Under the BVC model if a cell is shown to have a field along the South wall of a square environment then its likely firing position in a diamond or circular environment can be determined (see Figure 9). One of the predictions of the BVC model is that place cells will tend to produce fields that are oriented parallel to the walls of an environment. In general this appears to be the case: the place fields of cells recorded in circular arenas are crescent shaped and extending and environment results in extension of place fields (Muller and Kubie, 1987;O'Keefe and Burgess, 1996). The clearest prediction of the model is that the insertion of new barriers into an environment will result in a doubling of place fields. This has been seen in several experiments. Skaggs

& McNaughton (1998) saw cells with very similar fields in two halves of an interconnected environment, and Lever et al., (Lever et al., 1999) saw field doubling following the introduction of a new barrier into an environment. More recently, Barry et al., saw field doubling in 3/10 cells following barrier insertion, on or field shift responses in three others, two unaffected cells and 1 that moved away from the barrier (Barry et al., 2006)

Part of the utility of the BVC model is that it unambiguously states the nature of the inputs to place cells; they are derived directly from the boundaries/ barriers of an environment. This allows clear predictions to be made based on alterations in the geometric properties of an environment. One of the clearest predictions from the BVC model is the formation of double fields in response to barrier insertion. This is exactly what was seen with Barry et al., (2006) following insertion of a north-south barrier (see above). However, as the model stands, it cannot, without additional assumptions, explain the patterns of firing seen following non-geometric alterations. The formulation of the model as described above also cannot account for plastic changes that occur over many weeks of exposure to an environment (see for e.g. (Lever et al., 2002b). Furthermore, the hippocampus also has an increasingly well-documented role in the non-spatial features of a task or environment. Of particular relevance to the current discussion is the well documented role of the hippocampus in the representation of environmental context.

2.5. Context

As stated previously hippocampal place cells are known to respond to more than just the spatial properties of an environment. For example, changes in the

colour of a salient cue card (Bostock et al., 1991) and the odour of an environment (Anderson and Jeffery, 2003) have all been observed to cause changes in the responses of place cells. Because of the importance of place cells in defining current location, the issue of how they respond to context and the question of how context might be represented is of critical importance in understanding the hippocampal representation of the environment.

There have been several important theoretical accounts of hippocampal function in terms of its role in the processing of context (Nadel and Willner, 1980;O'Reilly and Rudy, 2001;Redish, 2001) These accounts vary in terms of their emphasis on the exact role of context, some focusing more on the spatial nature of the signal being processed (Nadel and Willner, 1980), others on a more general conjunctive role for the hippocampus (Rudy and O'Reilly, 1999). A more general purpose function such as this has been used to bridge the gap between the experimental findings in animals and humans (O'Reilly and Rudy, 2001;Redish, 2001).

The term context is widely used in the literature but is infrequently defined. Nadel & Willner's (1981) characterisation of context invoked a representation that was derived from cognitive map theory. They argued that the representation of context was defined by the stimuli present in the space described by the cognitive mapping system. An important distinction was drawn between discrete, foreground stimuli and the less pre-potent background stimuli. Contexts were conceived as existing in a hierarchical relationship with such discrete foreground cues. However, describing a cue as either fore- or background is not straightforward:

> "...contexts, like places, are not mere cues, or fixed aggregates of cues, to be treated as an X in a learning equation. Rather, contexts are overdetermined by the cues they contain, any of which could leap from the background into the foreground when the occasion arises." (p.227)

This conception of context stands in contrast to previous thinking of context as simply another discrete variable in an equation such as a conditioned stimulus (Rescorla and Wagner, 1972). Instead, an integrated concept of context was proposed that is internally represented and dependent on the hippocampal cognitive mapping system. Context was taken as something instantiated at a neural level rather than having correspondence to something "real" in the external world (see also (Jeffery et al., 2004;O'Keefe and Nadel, 1978).

Nadel & Willner's formulation of context deals with what they called "environmental context" (p.222). Their account emphasises the role of context in novelty detection and in place learning strategies. By localising the representation of context to the hippocampus and the cognitive mapping system, context is depicted as something that is internal to the organism. Indeed "intra-head" variables can also change the context in which an organism finds itself. Therefore internal variables can influence the behavioural decisions an animal makes. For example, Kennedy & Shapiro (2004) trained rats to approach different non-spatial goal objects based on their current internal motivational state (either hunger or thirst) (Kennedy and

Shapiro, 2004). This task was found to be hippocampal-dependent as hippocampal lesions severely impaired performance. Therefore the hippocampus is important for utilising internal as well as external contextual information. This suggests that the concept of context is richer than merely a system dependent on metric information pertaining to physical environmental features as suggested by the cognitive mapping hypothesis.

The proposal that the hippocampus is needed to represent context has since received considerable experimental support. Some of the strongest evidence comes from the fear conditioning literature (Kim and Fanselow, 1992;Phillips and LeDoux, 1992). In a typical context conditioning paradigm an animal is placed in a conditioning chamber and allowed to explore for several minutes. After this initial exploration period, an innocuous stimulus such as a tone is paired with an aversive one, such as an electric shock. Subsequent presentations of the tone and/ or the chamber where the conditioning took place are sufficient to elicit a variety of fear responses. Initially the rat shows strong locomotor activity that gradually gives way to a profound immobility called freezing. The most parsimonious account of this is in terms of Pavlovian conditioning (Pavlov, 1927). In the example described above the shock is equivalent to the unconditioned stimulus (US), the unconditioned response (UR) to the shock is the vigorous activity, the freezing is the conditioned response (CR) and the conditioned stimulus (CS) that produces the CR can be seen as either the constellation of cues that makes up the chamber (i.e. the context) or the tone itself. Conditioning to the tone is called classical conditioning, conditioning to the chamber independently of the tone is called context conditioning (Wiltgen and Fanselow, 2003).

Context and tone conditioning appear to rely on the integrity of different brain areas. The discovery that conditioning to a tone and to a context are sensitive to lesions of different brain areas came from two independent laboratories at the same time. Amygdala lesions affect both types of conditioning, hippocampal lesions impact context conditioning alone (Kim and Fanselow, 1992;Phillips and LeDoux, 1992). More specifically, pre-training electrolytic lesions of the hippocampus impair context conditioning but spare conditioning to the tone (Phillips and LeDoux, 1992). This suggests that the hippocampus is required for the acquisition of context fear conditioning. Furthermore, if the training to lesion interval in context conditioning paradigms is systematically varied then it is possible to see a pattern that is comparable to retrograde amnesia (Kim and Fanselow, 1992).

Deficits in the acquisition of context conditioning are seen when the delay between placement into the conditioning chamber and the administration of the shock is varied. Fanselow (1986) found that if this delay was less than about 40-60 seconds then freezing responses to the context were greatly attenuated (Fanselow, 1986). That pre-exposure to a context enhances context conditioning is unusual as pre-exposure to a stimulus normally reduces subsequent learning about that stimulus, a phenomenon called latent inhibition (Lubow and Moore, 1959). Such a pre-exposure requirement is not necessary for conditioning to the tone; administration of the shock within about 5-30 seconds of presentation of the tone is enough to achieve a robust conditioning effect. The deficit seen with short placement to shock intervals in context conditioning has been called the immediate shock deficit (ISD). It has led to the suggestion that the period between placement in the conditioning chamber

and administration of the shock is necessary for an integrated, configural representation of context to form (Fanselow, 1986;Fanselow, 1990). A configural representation describes the ability to associate an item with its context. A hippocampal dependent example of a configural representation is transverse patterning (Alvarado and Rudy, 1995b;Alvarado and Rudy, 1995a). Here three stimuli (A, B and C) are presented in a pair-wise, forced choice paradigm. The pairs and their rewards are $A^{+}B^{-}$, $B^{+}C^{-}$, $C^{+}A^{-}$. These choices are arranged so that that an elemental strategy cannot be used to solve the task. Instead the reward is dependent on the correct combinations of stimuli.

Such representations have variously been called unitary representations (Fanselow et al., 1993), configural associations (Rudy and Sutherland, 1989) or conjunctive representations (Rudy and O'Reilly, 2001). The elements that constitute a representation of context can be divided into the independent features that can separately enter into an association with an event, and a more holistic, integrated representation that encodes the cooccurrence of these features. Nadel & Willner map these two different representations to different brain areas – the independent features are represented in the neocortex and the integrated representation requires the interaction of the cortex with the hippocampus (Nadel and Willner, 1980). Rudy & O'Reilly (1999, 2001) discriminate between two different types of configural processing, one that requires the subject to pay explicit attention to the contingencies that define a problem, and a different type that occurs automatically as a consequence of an organism actively exploring its environment (Rudy and O'Reilly, 1999;Rudy and O'Reilly, 2001). Furthermore they specifically associate the latter form with the hippocampus. Conjunctive representations require the integration of

multiple, different sources of information into a coherent whole. The experimental evidence presented later supports the view that hippocampal place cells are able to integrate at least two different sources of information to form place fields.

It is evident that the hippocampus is critically involved in the processing of context. A context can be seen to consist of variables both external and internal to an animal which can be used to guide behavioural responses. Unlike other forms of learning, context conditioning requires substantial time to develop, as evidenced by the immediate shock deficit. Lesioning the hippocampus results in deficits in conditioning to a context and not a tone. Therefore a holistic, integrated representation of context depends on the integrity of the hippocampus. The experimental work described later examines, as separately as possible, the influence of two different sources of information important for driving place cell responses. Motivated by predictions from the BVC model, the influence of geometry is explored in a specially designed environment that allowed the sequential removal and extension of boundaries. Based in part on the interesting observation of learning-related changes in place cell activity seen following the extension of environmental boundaries, the final experiment examines the influence of context. The particular questions asked here relate to whether a learnt discrimination acquired by place cells in one context can transfer to a novel context. The findings from the experiments on geometric and contextual inputs are then incorporated into a modified version of the BVC model.

2.6. Conclusion

From the foregoing it is apparent that the hippocampus is pivotal in learning and memory, the processing of spatial relationships and the acquisition of context representations. The activity of individual cells in the hippocampus can provide insight into the nature of how these different types of processing are integrated into a coherent representation. Moreover, the manner in which such a coherent representation breaks down or reorganises (i.e. remaps) in response to alterations of the environment can further elucidate how such a representation is organised. All of the work carried out for this thesis involved changes to the geometric and contextual features of an animals' environment. Frequently this resulted in a remapping of one type or another occurring. The nature of the remapping that occurred following changes to the geometric and contextual properties of an environment provided important information regarding the afferent input arriving at CA1 place cells.

As was seen with the discussion of hippocampal anatomy, the hippocampus has a highly stereotyped structure, which begs the question, what, if any, structure underlies such function? If it exists, how does this function relate to the processes described above? Investigations of the electrophysiological activity of cells in the hippocampus have provided insight into these questions.

3. General Methods

3.1. Subjects and Housing

All procedures carried out in this thesis were approved by the Home Office, subject to the restrictions and provisions contained in the Animals (Scientific Procedures) Act of 1986. The animals used in this study were all male Lister Hooded rats (250-400g), obtained from the university breeding colony. Prior to surgery the rats were housed in groups of 4 with free access to food and water. Following surgery rats were housed singly with restricted access to food and *ad libitum* access to water. Initially rats were provided with food sufficient to reduce them to 85-90% of their free-feeding weight; following this they were given enough food to increase their weight by ~4g per week. Rats were maintained on a 11:11 light: dark cycle with half lights from 7am to 8am (simulated dawn) and from 7pm to 8pm (simulated dusk).

3.2. Electrodes and microdrives

Four tetrodes (Recce and O'Keefe, 1989) were each constructed from four interwound 25 μm diameter platinum-iridium wires (California Fine Wire, USA). The tetrodes were held in a microdrive assembly (Axona Ltd., St. Albans, UK) that allowed them to be lowered or raised with one full turn of the screw equal to an increment of 200 μm dorso-ventrally. Just prior to commencement of surgery, three of the tetrodes were cut level with each other; the other was staggered back by approximately 1 mm. This electrode would act as a reference whilst the other tetrodes recorded hippocampal cell responses.

3.3. Surgical Procedures

All of the animals used in this thesis underwent the same surgical procedure with only small changes in the anaesthetic regime employed. Before the start of any behavioural training all animals were surgically implanted with movable microelectrodes in order record multiple neurons. For Experiment 3 anaesthesia was induced in all animals with 0.2mL midazolam and fentanyl/ fluanisone (2.7mL/ kg intraperitoneal injection) and maintained with isoflurane and oxygen (2.5-4L/ min). The animals included in Experiment 1 and Experiment 2 were induced with isoflurane and oxygen (2.5-4L/ min). After a surgical level of anaesthesia was achieved, animals were placed in a stereotaxic frame with lambda and bregma in the horizontal plane. The eyes were covered with Vaseline to prevent corneal damage during the operation. Animals in Experiment 3 were covered with bubble-foam to help prevent heat loss, whereas animals in Experiment 1 and Experiment 2 had a heating pad placed under their bodies to maintain body temperature. All rats were monitored via frequent inspection of respiration and reflexes throughout the surgical procedure to ensure a satisfactory level of anaesthesia was maintained.

The scalp was incised with a longitudinal cut and retracted to expose the skull surface. The surface of the skull was then thoroughly cleaned so a clear view of the surface was achieved. Seven 1-mm burr holes were then drilled through the skull for placement of jeweller's screws to hold the microdrive assembly in place. One screw had a ground wire soldered to it so that the rat could be electrically grounded. A 2-mm trephine hole was then drilled above the right hippocampus at stereotaxic coordinates 3.8mm posterior and 2.5mm lateral to

bregma. The dura was then retracted to expose the cortical surface and the electrodes were introduced to a depth of 1.5mm from the surface of the dura. A metallic sleeve was then pulled down over the remaining exposed wires. Dental acrylic was applied to secure the microdrive assembly in place on the skull. Aureomycin (chlortetracycline hydrochloride) antibiotic powder was applied around the edges of the assembly and 0.1mL enrofloxacin (subcutaneously) and 0.1mL buprenorphine (intramuscularly) was given postoperatively and the animal was placed in a cage to recover. All animals were periodically monitored until they recovered. Animals in the second and third experiments were also given 2ml enrofloxacin in 500ml water as a prophylactic for no longer than seven days and also 1ml of Vetergesic dissolved in one cube of Rowntree's (Rowntree's, UK) strawberry flavoured jelly for pain relief for no longer than two days postoperatively (Flecknell et al 1999). All animals were given at least 1 week to recover following surgery.

3.4. Single-unit recording

Animals were handled regularly for at least two days after the one-week recovery period prior to commencement of recording. On recording days, the rats were connected to the recording equipment (Dacq, Axona Ltd., St. Albans, UK) via lightweight wires and a socket that connected to the microdrive plug. The potentials recorded on each of the 16 electrodes were passed through RC-coupled, unity-gain operational amplifiers mounted on the rat's head, and led to multi-channel recording equipment (Axona Ltd., St. Albans, UK), where the signal was amplified and filtered. For unit recording the signal was amplified 25000-40000 times and bandpass filtered (500 Hz – 7 kHz). Activity on each channel could be visualized by means of a single unit

oscilloscope display and listened to by means of an audio amplifier. Each of the four wires of one tetrode was recorded differentially with respect to one of the wires of another tetrode. One of the channels served to record an EEG signal. Prior to the start of recordings the tetrodes were driven down in steps of 25-200 μm daily until hippocampal "ripples" could be seen. As mentioned in the Neurophysiology section ripples reflect the synchronous bursting activity of large numbers of cells and are a reliable indicator of proximity to the pyramidal cell layer (O'Keefe and Nadel, 1978). During this period the rat was kept in an elevated holding area within the experimental room. Then the electrodes were lowered in small, 25-50 μm steps until hippocampal complex spiking cells were identified. Position was captured via a small LED on the head-stage assembly that was tracked by a video camera in the ceiling directly above the midpoint of the area in which the experiment was conducted. For Experiment 3 this resolved to the midpoint of the two boxes (see Experiment 3 Methods); for Experiment 1 and Experiment 2 this was the middle of the experimental room (see Experiment 1 Methods and Experiment 2 Methods). The position of the LED (~2cm above the rat's head, directly above the implant) was captured and converted into two camera coordinates. Each channel was monitored every 20 μs, and 50 points per channel were sampled whenever the signal on any of the four channels exceeded an empirically pre-determined threshold. Each presumptive spike event was stored on hard disk along with the position of the animal and the time since the start of the recording, and recorded on disk with the unit recordings for later off-line analysis. Cells were recorded until no more place cells could be isolated from that electrode location, at which point the microdrive was lowered another 25-

50 μm until further cells could be discriminated.

3.5. Place field measures

Data analysis was performed off-line using cluster-cutting software ("Tint", Axona Ltd., St. Albans, UK). The path of the rat was smoothed using a boxcar algorithm with a boxcar width of 400ms. Collected waveforms were distinguished by plotting the peak-to-trough amplitude of one electrode against that of each of the other three on a series of scatter-plots (see Figure 10). Because the extracellularly recorded spike amplitude decreases with distance and each wire of a single tetrode is a different distance from a given cell, each wire "sees" a different amplitude spike. Therefore it is possible to estimate the "distance" of recorded neurons from a tetrode wire by triangulation with the other wires on that tetrode. Using this technique spikes belonging to a single cell typically appear as clusters. These clusters were then separated by hand or by an automatic cutting algorithm (based on the manual cut) and these collections of spikes were assigned different cell numbers.

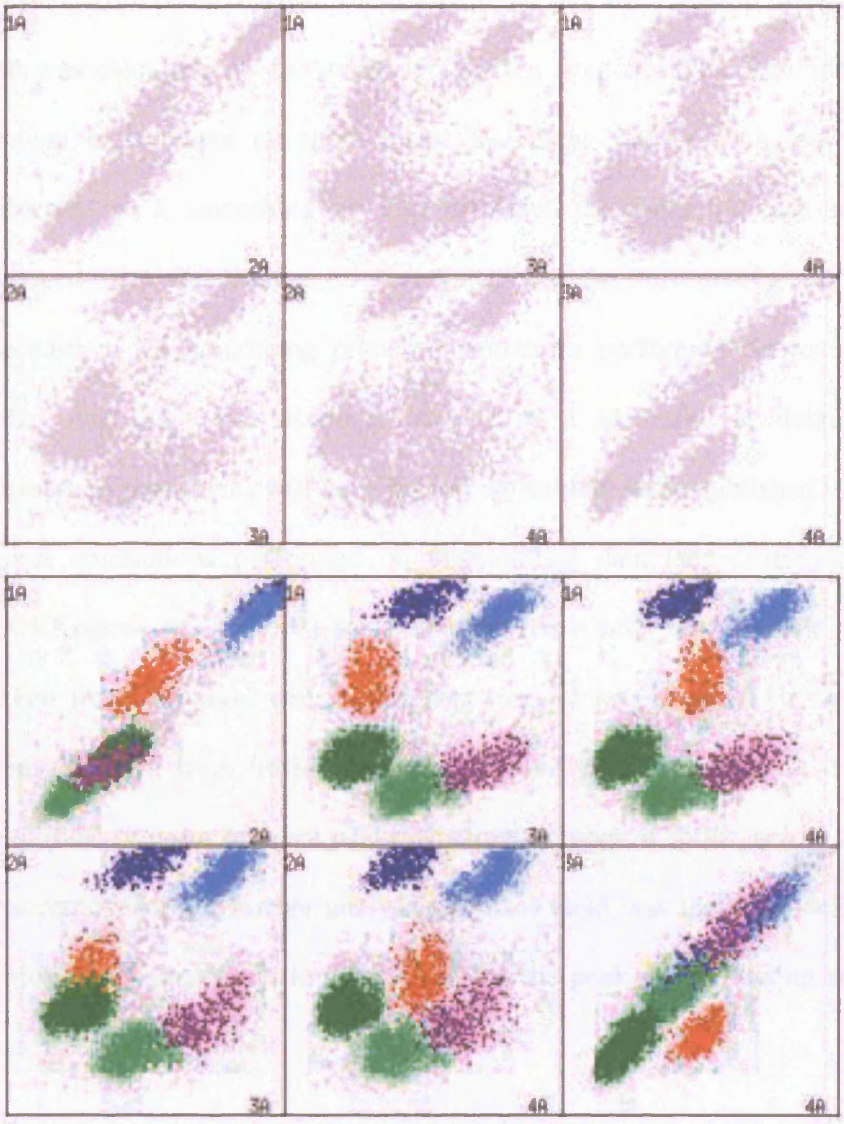


Figure 10. Collected action potentials from neurons in CA1 plotted as a series of peak-to-trough plots (in microvolts). Numbers at top left and bottom right of each plot indicate which wire of a tetrode is being plotted (for example the top left panel of each main panel shows the peak-to-trough amplitude of wire 1A plotted against the amplitude recorded on wire 2A; as there are 4 wires in each tetrode there are a total of 6 possible comparisons/ plots). Although not displayed for clarity, units on the x and y axes for each plot are in microvolts. Coloured "clusters" of spikes in the lower half of the figure correspond to different cell assignments.

To determine where in the environment the cell's place field was located, the camera viewing area was divided into a 64x64 grid, each point of which was

located at the centre of a ~2.25cm square bin. For each bin the firing rate for a cell was calculated as the number of spikes fired in that bin divided by the amount of time the rat spent there. The firing rate in each bin was then subjected to a smoothing process in which the value in each pixel was replaced with the average of that value and the surrounding eight pixels. Because of the smoothing process correlations performed between pairs of firing rate maps (see Experiments 1,2 & 3 Methods for details of the correlation procedure) will have higher values than some published values that report correlations performed on unsmoothed data (see (Agnihotri et al., 2004;Kentros et al., 2004) for examples). Any cells with a peak frequency (taken from the pixel with the highest rate) of less than 1.0 Hz in any trial were excluded from further analysis. A poorly defined place field was one with three or more separate peaks or a total number of spikes below thirty and was removed from further analysis. A place field was therefore defined as a region of location-specific firing in which the peak rate following smoothing was greater than 1.0 Hz.

3.6. Histology

At the end of each experiment, animals were transcardially perfused with saline, followed by a 4% paraformaldehyde (PFA) solution. The brains were removed and stored in a 4% PFA solution for at least a week before sectioning began. Brains were sectioned at both 40 μm and 50 μm. All sections were stained with a cresyl violet method to verify electrode placement. The cresyl violet method involved stepping the sectioned brain material through an ascending alcohol series, a descending alcohol series, immersion in a cresyl violet solution, and finally another ascending alcohol series. Following this

the stained tissue was mounted on glass slides, cover-slipped and allowed to dry. Electrodes in all rats were confirmed to have been placed in region CA1 of the hippocampus. No electrode penetrations were confirmed to have progressed as far as CA3.

4. Experiment 1

4.1. Introduction

The current experiment, and the one following it, explores the nature of geometric inputs to place cells in more detail. By examining the effect of removing environmental boundaries on place cell activity this experiment sought to test predictions derived from the BVC model (Burgess et al., 2000;Hartley et al., 2000). The specific questions addressed include the minimal requirements in terms of environmental stimuli that can drive the formation of place fields and whether place fields can be controlled by single, point-like cues. By decomposing the boundaries that constitute an environment it was hoped that the BVC inputs arriving at individual place cells could be similarly decomposed. If the boundaries to an environment were decomposed, thereby removing or silencing putative BVC input to place cells, would the place fields themselves exhibit a similar decomposition evidenced by reductions in the coherence or stability of place fields? Therefore, one of the aims of the current experiment was to create a recording environment that was "boundary-less" in terms of the walls that made up the room. It was hoped this would allow the influence of boundaries to be investigated without the confounding influence of other, uncontrolled boundaries. Another aim was to investigate if the minimum theoretical requirements for accurate self-localisation in space would also enable place cells to form coherent, "normal" place fields. In theory, a point-like landmark and a directional cue provide enough information to specify a point in two-dimensional space. If such a denuded environment could support "normal" place cell firing then this would provide insight into the minimal requirements

for place fields to form. What follows is the rationale for these manipulations and previous findings that have examined the influence of objects and boundaries on place cell activity.

The majority of place cell experiments involve placing an animal in an enclosure of some type and allowing the animal to forage for food or seek reward whilst the activity of the cells is recorded. Limiting the animals' locomotor activity to an enclosure allows an experimenter a high degree of control over the space sampled by the animal during the trial and facilitates subsequent analysis. Perhaps more importantly, it also allows the experimenter control of the spatial features the animal is exposed to. However, such experiments can be criticised for a lack of ecological validity. If, as in many experiments, a high-walled arena is used, then this is equivalent to having a room within a room; the arena itself is within the experimental room. This can be compounded when the arena itself is placed within a so-called cue-controlled environment. Such an environment consists of a curtained off area (typically about 2-3m in diameter) outside of which is located the experimenter and all the relevant recording equipment (sometimes this is removed to an adjacent room). This essentially equates to having a room within a room within a room (one is reminded of Russian dolls).

Other issues of ecological validity were recognised by O'Keefe & Nadel in The Hippocampus as a Cognitive Map (1971); allowing an animal to freely forage in an environment is more "neuroethological" approach than training an animal to perform a complicated operant response, something it is highly unlikely to be required to do in its natural habitat. However, due to the

requirement of a tether that transmits the electrical activity occurring in the region of brain tissue at the end of the electrodes to the recording equipment, it is not yet possible to record from an animal that is completely free to move. The tether is just that; a tether that constrains the possible degrees of freedom. Despite these considerations it is still possible to make steps towards a more naturalistic situation where the animal could freely forage for food in an open, unconstrained environment*. In such an open environment it is possible to more purely assess the influence of boundaries and objects on place cell firing.

Many place cell experiments have investigated the influence of what have been variously called intra-maze, local or proximal cues versus the influence of extra-maze or distal cues. Examples of local cues include differently textured and scented floor surfaces, as well as different visual patterns on the floor surfaces; local cues are typically features of the environment physically accessible to the animal. In contrast, distal cues consist of large, bold objects that are supposedly distinct to the animal, that are not physically accessible as they remain outside of the arena the animal moves around in. Examples of distal cues include hat-stands (with or without lab coats attached), large polystyrene objects and cue cards attached to the black curtains of a cue-controlled environment. Alternatively, the cue controlled environment can be removed and the distal cues can consist of the furniture and apparatus present in the experimental room (as with Experiment 3). A third type of cue can be added to this list consists of distinct objects placed within the recording arena. Although these are physically accessible as with the local cues, they differ in

* Typically, an open-*field* environment is roughly the dimensions of those used in Experiment 3. It has discrete boundaries that prevent the animal from escaping. Alternatively the arena can have no walls and prevent the animal from escape by elevating it above floor level.

the sense that local cues, as defined above, extend across (and help to *define*) extended surfaces. Cues placed within an arena are more "point-like" in nature; they are discrete objects as opposed to delimiters of boundaries and an animal typically has 360 ° access to them.

Studies that have examined the influence of intra-apparatus objects and barriers on place cell activity have generated several findings relevant to the current experiment. The first examination of the effect of objects on place cell activity can be seen with Muller & Kubie (1987). Inserting a barrier in the region of an established place field abolished the place field on subsequent trials. This field attenuation effect was limited to cells with fields near the barrier (a point examined in more detail below). Interestingly, one place field of ten cells recorded during the barrier experiment was seen to expand when a barrier was placed as to bisect the place field. The authors viewed this response as evidence that the place cell had reorganised its firing field to accommodate the spatial changes via a remapping. Whether this somewhat idiosyncratic response was a general property of the place cell representation is difficult to say as no information is provided as to whether this cell was recorded in isolation or with other cells. It is therefore difficult to say how the representation as a whole was treating the barrier.

The influence of extended surfaces on place cells has been elucidated in more detail recently in a study by Rivard et al., (2004). As with the Muller & Kubie experiment above, place cells were recorded in a circular apparatus with a barrier in various positions in the arena. The authors claimed to have found a new class of place cell that signals proximity to the barrier. Place cells that

had fields near to the barrier (~10cm) moved their fields with the barrier when it was rotated about the arena centre in 45° increments. On the other hand, cells whose fields were further away from the barrier, closer to the main arena wall, were not affected by such rotations. Furthermore, the fields of these cells were also shown to move with the barrier when it was translated across the arena (i.e. if the barrier was at a 6:00 o'clock position, then it was translated to a 12:00 o'clock position). Most importantly, when these cells were recorded in a different environment (sufficiently different to induce a complete remapping) the cells still discharged at the barrier (Rivard et al., 2004). In addition to these responses a third type of response was noted that suggested an interaction between the barrier and its location in the environment. These cells had fields with similar responses to the fields that were abolished when a barrier intersected them in the Muller & Kubie (1987) experiment.

Several other experiments have examined the influence of objects within an environment on place cell activity. As mentioned in the Introduction, Cressant et al. recorded cells in a circular environment that had three three-dimensional objects in it. These objects were placed in one of two different configurations. They were either grouped together in the middle of the arena or placed at the periphery of the arena against the environment walls. The main finding was that place cells were not controlled (in terms of rotational remapping) by the objects when they were in the centre of the arena, but were controlled by the objects when they were at the arena periphery.

Based on further work it appears that it is not just the geometric relationship

between the objects that determines if the objects control place fields (Cressant et al., 1997). Interestingly, the identity of the individual objects appears to be a relevant dimension. If the objects are placed in an equilateral triangle arrangement (as opposed to an isosceles triangle as in the original experiment) at the arena periphery, rotational control of place fields still occurs. Therefore the identity of the objects *can* assist with at least the rotational localisation of place fields (see below). However this cannot be the only information utilised to locate firing fields. If it was then fields should also be controlled by the objects when they are in the centre of the environment, provided they are rotated as a rigid set. Instead an interaction must have occurred between the individual identity and the location of the objects. A further possibility is that the head direction system could be responsible for discriminating objects and providing place cells with information that allows the localisation of their fields at the correct distance from the objects.

An experiment by Gothard et al., (1996) fractionated place cells into distinct classes based on their responses to manipulations of objects within an environment (Gothard et al., 1996). Here animals were trained to navigate to a food reward location indicated by two landmarks that remained fixed relative to each other. During performance of the task the start box was moved to a new location. The authors found that there were four different types of cell; i) "traditional" place cells with location-specific firing, ii) goal/ landmark cells that fired in the vicinity of the goal/ landmarks no matter where they were in the arena, iii) box-related cells that fired either as the rat was leaving or returning to the box, regardless of where in the arena it was and, iv) cells with

separate fields in more than one of these categories.

The second and third types of cell appear to encode location with respect to individual objects in the arena. This result can be contrasted to the cell behaviour seen with Cressant et al (1997, 1999). No separable categories of cells were observed in those experiments. It was proposed that this may have been because the objects in those experiments were behaviourally irrelevant to the animals[7]. However this proposal is not consistent with the findings of Rivard et al., as the barriers in that experiment were also behaviourally irrelevant. It is also possible that the failure to find cells classifiable as object-linked as with Gothard et al (1996) was due to the paucity of spatial information in the environment. With Gothard et al the start box and two landmarks were located in a high-walled circular arena that has many other cues located at its periphery. In contrast, the arena in the Cressant et al study was located within a curtained off environment with no cues attached. The same can be said of the Rivard et al., result as the circular arena used in that study had a prominent cue card attached to the wall. The presence of such a stable spatial cue may well be important for establishing the barrier as a good predictor of spatial location and therefore allow it to exert strong control over place cell activity. This is a point that will be returned to in the Discussion as it is possible that the presence of a prominent spatial cue may influence the stability and coherence of place fields.

Two sets of experiments have been described so far: those involving manipulations of boundaries within an environment (Muller and Kubie, 1987)

[7] The barriers *are* behaviourally relevant in terms of preventing the possible paths the animal could take, but not in the sense that they are used to guide a particular behavioural response.

and those manipulating objects within an environment (Rivard et al., 2004) or the relationships between goal locations and objects (Gothard et al., 1996). As mentioned above, an interesting question is exactly what constitutes a barrier or an object; objects are discrete entities occupying an almost point-like position in space whereas barriers/boundaries delimit space in an extended manner. Moreover, is it possible to blur the distinction between them, or change the classification of a barrier to an object or vice versa?

In order to begin addressing these issues the current experiment sought to determine the influence of the constituent parts of a box environment on place cell activity. The components of the square box included four walls and pillars at each corner that supported them. Each of the walls was transparent (the pillars were opaque) so the animal could view outside the box. The box itself was located within a room under conditions that attenuated as much as possible the animals view of and the potential for contact with the room walls. In order to achieve this, a novel reinforcement procedure was employed that prevented the animal from proceeding to the room walls. A weak directional cue was attached to one of the distant room walls. During an individual session, trials were run that involved gradually removing the walls of the box one at a time until only a single pillar remained. There were several aims of this manipulation. The first aim was to examine the effect of removing a single boundary from the environment. Would place cells exhibit the same firing pattern in the three-walled environment as in the bounded, four-walled environment? The second aim was to see if "normal", coherent place fields formed in such a reduced cue environment (see Figure 11). Third, following removal of the walls and the associated pillars, it was also of interest to ask

whether place cells would fire in locations far from the reduced walls or only near, or in, the reduced box. Last, it was of interest to see if the reduced local cue set could accurately control place field location. In order to test this, the remaining pillar was moved around the experimental room to see if place fields would follow the pillar.

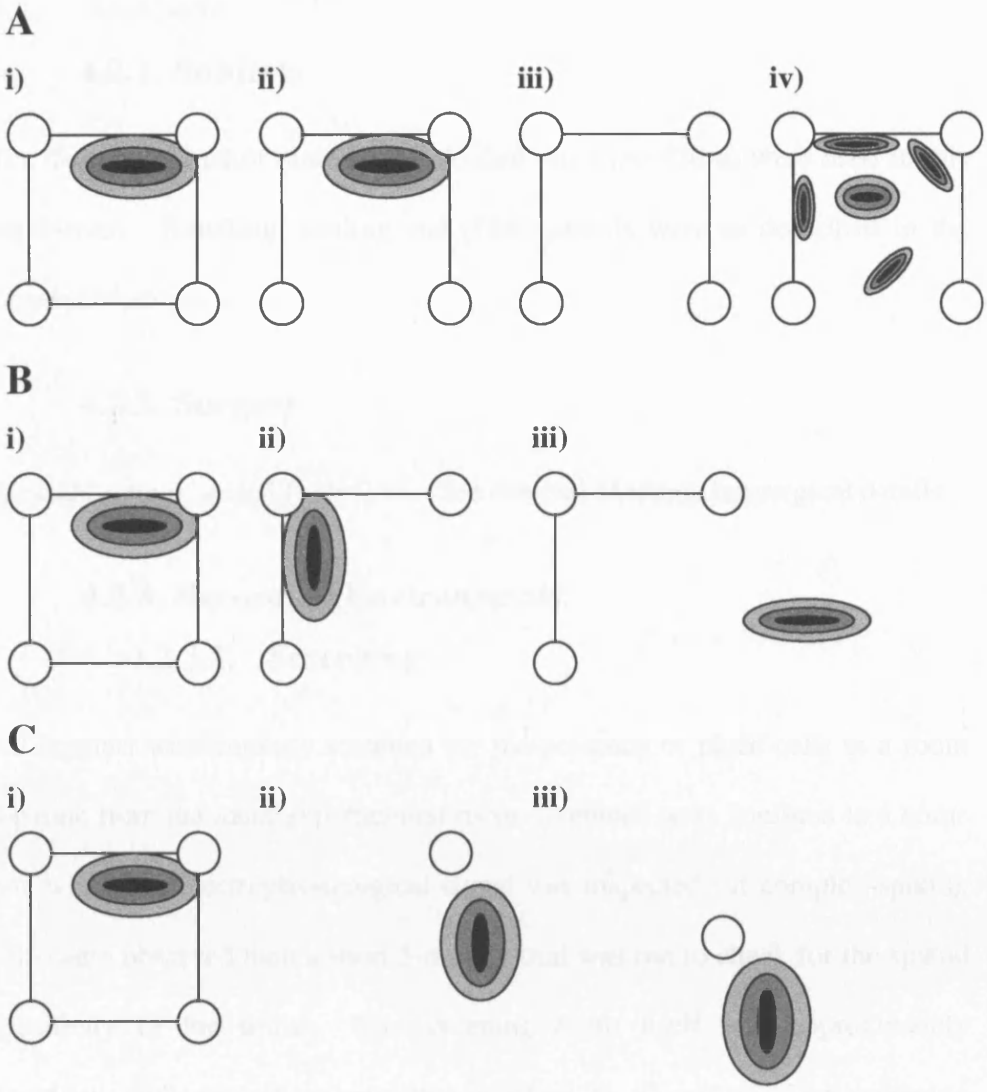


Figure 11. Aims and possible outcomes. A) The possible effects of removing a single boundary on place fields. i) In the standard bounded environment a place cell is active to the south of the north wall of the bounded environment. ii) Following removal of a single boundary the place field remains in the same location as in i). iii) An alternative possibility is that a remapping occurs. Here the cell becomes silent. iv) Another potential outcome is that place fields break down and decohere. B) In an attenuated environment place fields could either be localised near the remaining boundaries or separate from them. i) A place field similar to Ai. ii) The place field remaps and remains close to the remaining set of boundaries. iii) The place field here is coherent and distant from the remaining boundaries. C) Place field control by a single salient landmark. i) The place field is as in Ai). ii) Following reduction to the single pillar condition a coherent field is present to the South of the pillar. iii) When the pillar is moved around the experimental room, the place field moves with the pillar.

4.2. Methods

4.2.1. Subjects

The data from 2 adult male Lister Hooded rats (350-420 g) were used in this experiment. Handling, feeding and photo-periods were as described in the General Methods.

4.2.2. Surgery

All cells were recorded from CA1 – see General Methods for surgical details.

4.2.3. Recording Environments.

4.2.3.1. Screening

All animals were initially screened for the presence of place cells in a room separate from the main experimental room. Animals were confined to a home box whilst the electrophysiological signal was inspected. If complex-spiking cells were observed then a short 5-minute trial was run to check for the spatial specificity of the units. The screening room itself was approximately 4.5x4.5m with several salient cues attached to its walls. The screening environment was a black box constructed from cardboard of dimensions 70x70x34cm located in the centre of the screening room. The screening environment was elevated off the floor by approximately 35cm. After the 5-minute trial the data was inspected to check for the presence of place cells. Following the successful identification of place cells the rat was unplugged from the recording system, placed in an opaque box and transferred to the main recording room.

4.2.3.2. Main recording room

The main recording room was approximately 6.3x5.3m and had dark black floor-to-ceiling curtains attached to all walls such that the walls behind the curtains were not visible. The video camera used to capture the position of the rat was located in the centre of the ceiling. To the north of the camera was located the pre-amplifier, also attached to the ceiling, which was connected to the head-stage (attached to the rat); the data ribbon-cable was taped to the ceiling and led out of the room (to the main recording equipment in an adjacent room) via a hole that was drilled in the top-middle of the north wall. Just below this hole was located a loudspeaker which had cables also leading out through the hole. The loudspeaker was held in a pocket sewn into the curtains so that it was held between the curtain and the wall. The floor of the main recording room consisted of several layers of different material. First it was lined with aluminium foil which was electrically grounded in an attempt to remove as much unwanted electromagnetic noise as possible. Above this was a 5mm layer of rubber matting. Next was a layer of foam-board sheets (84x60cmx6mm). On top of this, the surface that the rat was in contact with, was a series of black-coloured A1-sized sheets of polypropylene. These polypropylene sheets were regularly wiped with ethanol to remove odours and randomly shuffled between each trial. A weak directional cue was provided in the form of a low-powered 5W bulb situated 1.5m above ground level in the middle of the North wall.

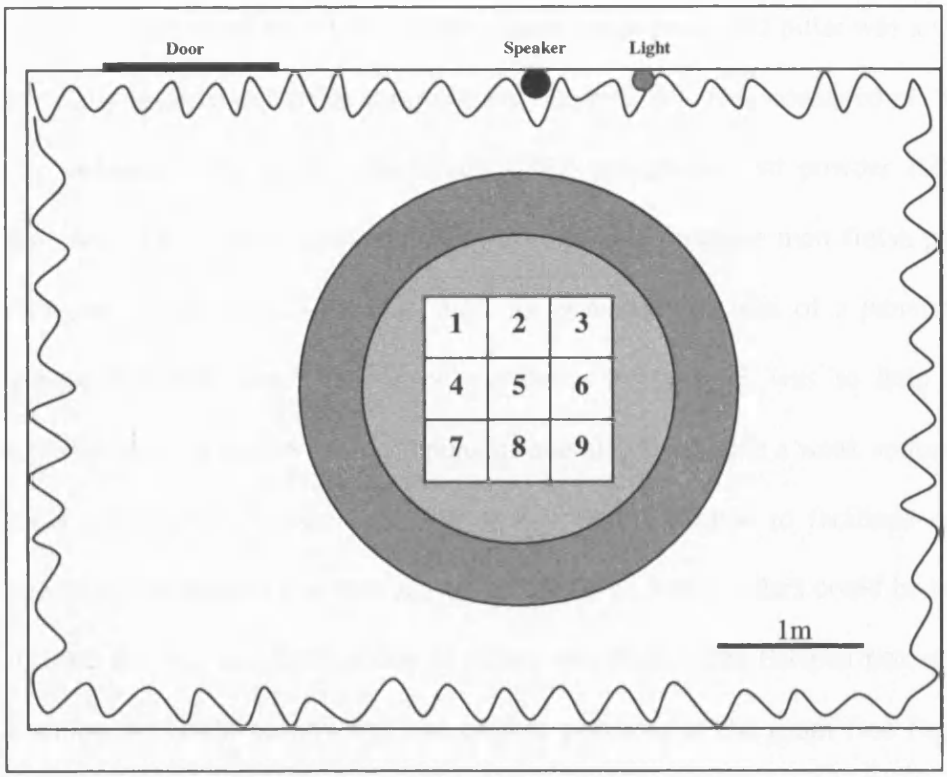


Figure 12. Main experimental room and possible positions local box could occupy. Numbers indicate 9 possible box positions (box was centred on each number). The inner, light grey circle shows the "silent" zone, the dark, grey circle the mild white noise zone. Locations outside the dark grey circle elicited the loud white noise. Wavy line at edge of room shows black curtain.

4.2.3.3. Local environment

Within the main recording room was located a box that consisted of four transparent Perspex panels connected to each other at their edges by four opaque polypropylene pillars. Each pillar was 60cm tall with a radius of 6cm. Two narrow channels were cut out of each pillar to allow the panels to be slotted into the pillars and give the whole apparatus rigidity. The channels were 25cm long and 80mm wide to accommodate the panels. Each panel was 75mm thick and 70cm long along the top edge (65cm along the bottom edge) with a notch cut out of each end to allow the panel to slide into a pillar.

Approximately 5cm above the bottom edge of each panel and pillar was a strip of "glow-in-the-dark" paint approximately 3cm thick. This consisted of 40% (by volume) Ultra Blue "Glow-in-the-dark" phosphorescent powder (Glow Inc., MD, USA) that was mixed with Ronseal polyurethane matt finish paint (Ronseal, Sheffield, UK). This strip ran around both sides of a panel and around the full circumference of a pillar. Its purpose was to help the experimenter locate the box/ components and also to provide a weak source of local illumination for the rat. The boxes were designed to facilitate easy assembly/ disassembly so that any combination of walls/ pillars could be used to form the box or combinations of pillars and walls. The Perspex box or its components could occupy any one of nine positions in the room (see Figure 12), with the centre of the box aligned with the given position. The distance between each position was half of the width of the local box (~32.5cm). When the box was reduced to its component parts the imaginary centre of the box was used to line up the components on any given position.

4.2.3.3.1. Spatial reinforcement procedure

In order to prevent the rat from coming into contact with the room walls a sound "barrier" was created that produced different levels of white noise depending on the rats location in the room. To this end, two imaginary concentric circles were drawn around the current box location, incursion into which resulted in the generation of white noise from a loudspeaker mounted on the North wall (see Figure 12). Incursion out of the inner circle produced a mild white noise (~85dB at the level of the rat in the middle of the room) following a 250ms delay. When the rat returned to the inner circle the white noise terminated after a 100ms delay. Incursion out of the outer circle

produced a loud white noise (~105dB) after a 250ms delay and reverted to the mild white noise following a return to within the outer circle after a 100ms delay. The purpose of the delays was to prevent the location of the edge of the sound zones serving as stable spatial cues that would allow the animal to localise itself in the environment.

Examples of the paths taken by animals as a result of this spatial reinforcement procedure can be seen in Figure 14. Following entry into the white noise area the animal takes a direct path back to the vicinity of the once described by the four walls of the box. It can also be seen that the animal spends the majority of the time of the trial in the area of the box.

4.2.4. Recording protocol

4.2.4.1. Trial structure

The standard trial consisted of a five minute recording period in the box with all walls and pillars present. This type of trial was always the first trial run at the start of a recording session, although it was not always run in the same start position at the beginning of a session. Following this, one of several different trials could be run; removal of one, two or three walls and removal of all walls to leave only a single pillar standing. These trials were fifteen minutes long as the rat had a much larger space to sample. Each of the different combinations could be positioned at any of the 9 different locations in the experimental room as indicated in Figure 12.

4.2.4.2. Handling details

At the start of each recording session the rat was brought into the main recording room in an opaque transport box, and the lights were turned off. The rat was then removed from the box and attached to the headstage; the experimenter illuminated the back of the implant with a narrow beam flashlight (Mag Instrument Inc., USA) to assist safe attachment. The rat was then placed back in the transport box and the lid was placed back on with a small gap to allow free movement of the headstage wires. The lights were then turned on and any relevant stimulus changes made. The experimenter then left the main recording room to prepare the recording equipment in an adjacent room. Once this was completed the experimenter re-entered the main recording room, turned the lights off, removed the rat from its transport box and placed it in the local environment. The recording session was started via

remote control as soon as the rat had been placed down.

The rat was always placed in the same position; aligned with and next to the North wall, facing East (or the equivalent position if the North wall was removed). The transport box was picked up and carried by the experimenter throughout the trial. As soon as the rat was placed in the local environment, recording was initiated via remote control. During the trial rice was dropped within and (if walls were removed) around, the local environment to encourage the rat to forage. When the trial had finished the rat was picked up from wherever it was in the room and placed back into the transport box. The box was always placed just south of the North wall of the local environment (i.e. where it was at the start of the trial). The lid was then placed on top of the box and the lights turned on. Whilst the rat was in the transport box the experimental room was cleaned by wiping the floor tiles with ethanol and the polypropylene A1 sheets were moved pseudo-randomly to new positions to scramble local olfactory cues/ markings. The walls and pillars that constituted the local recording environment were also randomised. In the first wall removal manipulation only a single wall was removed; this was hidden behind the curtains lining the walls of the experimental room. In the second and subsequent wall removal manipulations a wall and the adjoining pillar was removed. These were similarly hidden behind the curtains. When the final pillar was moved around the room it was moved into positions as though it was still connected to the box. Following completion of a wall/pillar removal manipulation the next trial was begun. At the end of the recording session, when the rat was still in the transport box, the lights were turned off, the rat was removed from the box and unplugged from the headstage, placed back in

the transport box and removed from the main recording room.

4.2.5. Single-unit recording

See General Methods for details of single-unit recording.

4.2.6. Place field measures

The same criteria outlined in the General Methods for including a cell in the analysis were applied in the current experiment. Several measures were used to assess the effect of environmental changes on place cell activity. These included;

i. The number of pixels visited by the animal during a trial

ii. The number of pixels in which a cell fired

iii. Place field size (expressed as a ratio of the total area covered by the animal during a trial)

iv. Coherence (Muller and Kubie, 1989)

v. Spatial information content (Skaggs et al., 1993)

vi. The number of action potentials fired by the cell and

vii. The overall rate of the cell for a trial.

Coherence is a correlation-based measure that compares the firing rate in a given pixel with the mean rate in the pixels surrounding it. Place cells with compact place fields will have high coherence scores and those with dispersed or "messy" place fields will have lower coherence scores. Spatial information

is a measure of the amount of information (in bits per spike) conveyed by the firing rate of a cell about the location of the animal. Correlation measures were also employed to assess the similarity of place fields between the various conditions. The correlations were calculated on place fields constructed/ assessed across four different types of analysis (see below). Any pixels that were unvisited in any map did not contribute to the correlation analysis.

Each of the above measures was assessed under four different types of analysis that were designed to look at the different possible reference frames the cells could potentially have used to organise their firing (Redish, 2001;Rosenzweig et al., 2003). The different types of analysis carried out were (see also Figure 13),

i. Room frame / whole room. This analysis examined data collected across the whole experimental room. When correlations were performed between firing rate maps in this analysis the maps were untreated i.e. they weren't realigned to one another as with other analyses (see below). Therefore this analysis was conducted with respect to a reference frame aligned to the room.

ii. Box frame/ whole room. This analysis also examined data collected across the whole experimental. Firing rate maps in this analysis *were* realigned to the central room position (position 5). If place cells were firing with respect to the box frame then correlations performed under this analysis would be expected to be high as the maps were realigned with respect to each other. For an example of the difference between this analysis and the room frame/ whole

room analysis, see Figure 19A & B.

iii. Room frame/ box only. This analysis only considered data collected when the rat was within the boundaries described by the walls of the box. Any data collected when the rat was outside the region described by the box was left out of this analysis. For example, if the box was in position 5 (the central position in the room) only data that was collected in an area equal to the size of the box centred on position 5 was entered into the analysis. Equally, if the box was moved to position 1 (North-West of position 5) then only data collected in an area equal to the size of the box centred on position 1 was entered into the analysis. Therefore the reference frame that this analysis examined was aligned to the local box.

iv. Box frame/ box only. This analysis also only considered data collected when the rat was within the boundaries described by the walls of the box. Additionally the firing rate maps in this analysis were realigned to the central room position (position 5) as with the box frame / whole room analysis. For an example of the difference between this analysis and the room frame / box only analysis, see Figure 21A & B.

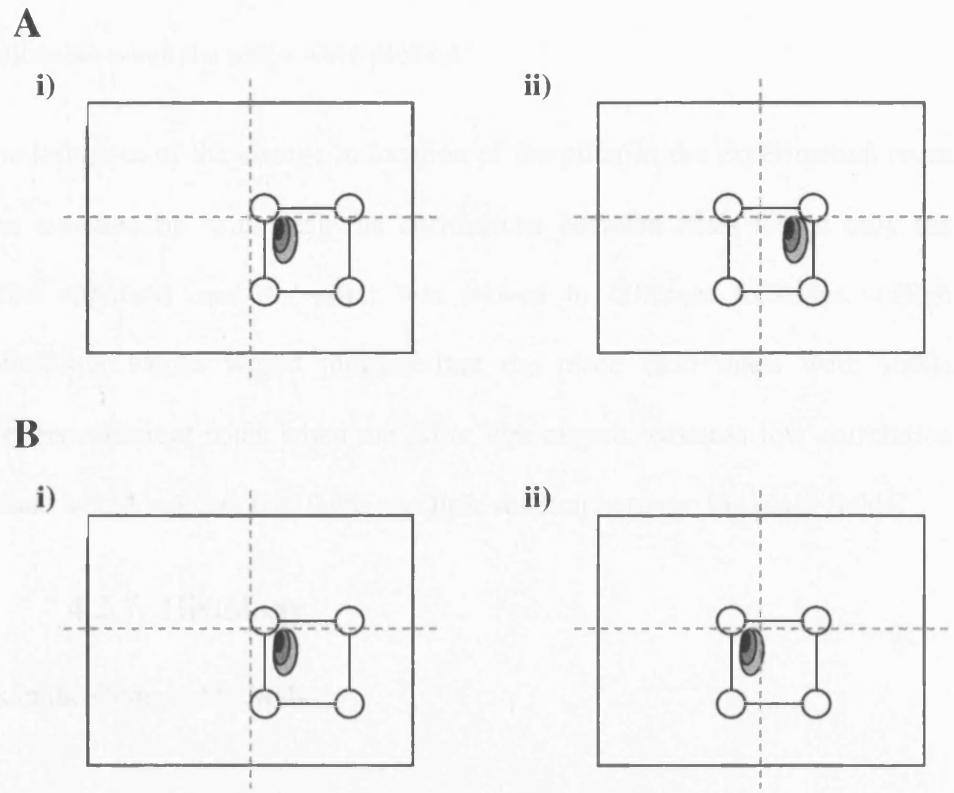


Figure 13. The different types of reference frames that place cells could utilise to orient their firing. A) A room-based reference frame. i) A place field is shown to the east of the west wall. ii) When the environment is moved to the west the place field remains in the same location with reference to the room as in i). B) A box-based reference frame. i) The same place field as in Ai). ii) When the box is moved to the west the place field maintains its relationship to the box and not the room.

The two types of box frame analyses were performed to see if place fields in these conditions moved around the room concomitantly with the local box ensemble (or parts thereof). The box vs. room analyses were conducted because of non-homogeneous spatial sampling outside the local box. The area inside the local box was the best sampled area in the room and therefore possessed the highest resolution sampling. An additional reason for the box vs. room analyses was due to high "out-box" firing that was observed during pilot experiments. Because the firing rate maps are scaled according to the peak rate across the whole trial, it was possible that any firing that occurred in

the box would be attenuated by high firing rates outside the box and not be noticeable when the maps were plotted.

The influence of the change in location of the pillar in the experimental room was assessed by examining the correlations between trials where only the pillar remained *and* the pillar was moved to different locations. High correlation values would indicate that the place field maps were stable between different trials when the pillar was moved, whereas low correlation values would indicate that there was little relation between the place fields.

4.2.7. Histology

As in the General Methods.

4.3. Results

4.3.1. Behavioural observations

As soon as the first wall was removed from the local environment the rats had access to the rest of the experimental room. Following a wall removal, rats left the region bounded by the remaining walls and explored the area immediately outside the local box (see Figure 14). The effect of the white noise "virtual" boundary can be seen in Figure 14. Rats appeared to rapidly learn to come back to the vicinity of the local box in order to turn the white noise off. Examination of the paths of the rats shows that the sound barrier was very effective in preventing them from coming into contact with the experimental room walls.

4.3.2. Histology

Electrodes in all rats were confirmed to have been placed in region CA1 of the hippocampus. This was additionally confirmed by electrophysiological criteria (see General Methods).

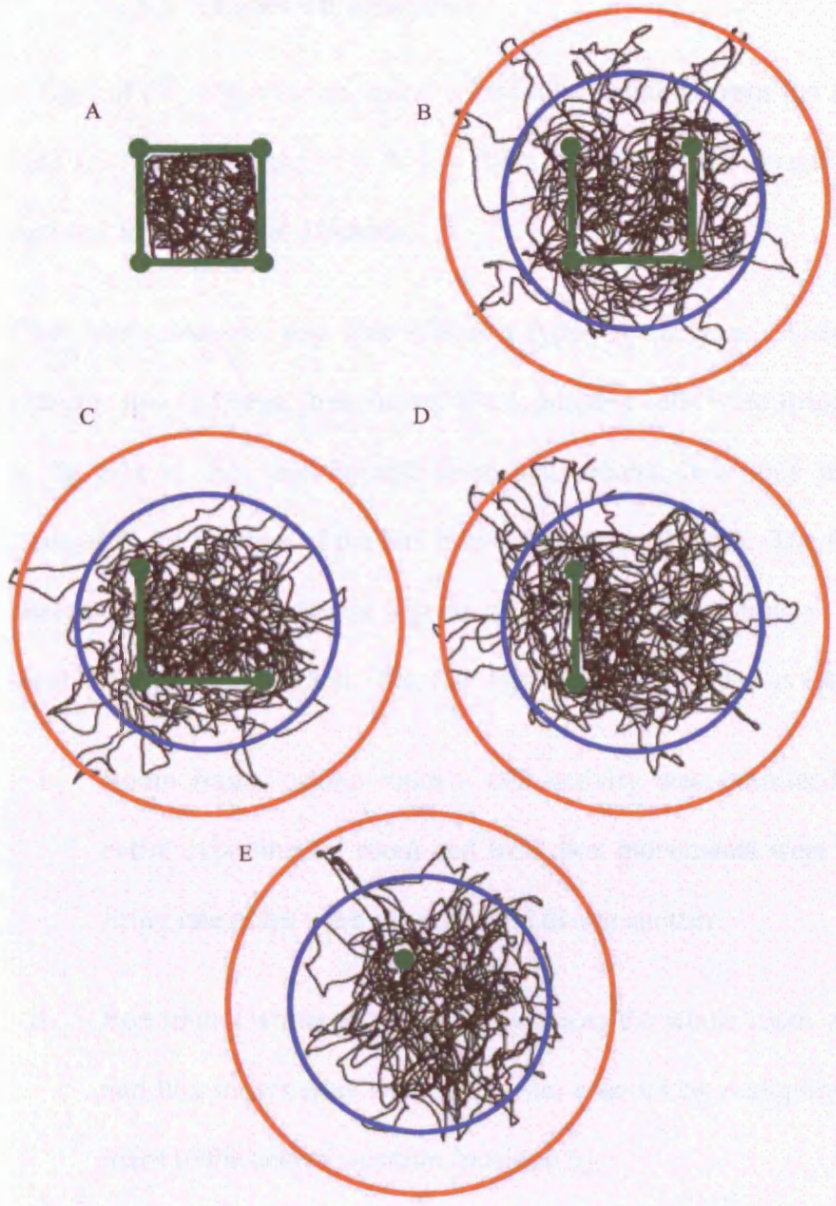


Figure 14. Paths from a single session for all four five types of wall removal trial. A) Four walled trial, B) the North (top) wall is removed, C) the North and East walls are removed, D) the West wall remains and, E) the North-West pillar is left. The inner blue circle represents the minimum radius at which the quiet white noise would sound and the outer red circle represents the minimum radius at which the loud white noise would sound. Initiation of the quiet white sound barrier was randomly variable between the two circles following the animas re-entry to the quiet zone (i.e. within the blue circle).

4.3.3. Place cell analyses

A total of 33 cells were recorded across all conditions from the two animals used in this experiment (n = 5, n = 28) that satisfied the acceptance criteria outlined in the General Methods.

Cells were assessed with four different types of analyses. These aimed to examine two different phenomena. First, whether cells were firing in relation to the box or the experimental room and second, how they responded to changes in the location of the box in the experimental room. The four types of analysis focused on different aspects of the environment and/ or whether the local box was moved or not. The four types of analysis were as follows:

i. Room frame/ whole room – cell activity was examined across the entire experimental room and local box movements were ignored i.e. firing rate maps were not realigned to one another.

ii. Box frame/ whole room – activity across the whole room was assessed and box movements were taken into account by realigning firing rate maps to the central position (position 5).

iii. Room frame/ box only – only cell activity that occurred within the boundaries described by the local box was considered, ignoring local box movements (maps were *not* realigned).

iv. Box frame/ box only - the same area was considered as in i) and box movements were taken into account by realigning firing rate maps to the central position (position 5).

Before these analyses were conducted, place fields were compared between the first two trials conducted in a session. The first trial was always a full, four walled trial and the second was always with a single wall removed. Comparing these two trials gives an indication of the effects of removing a single boundary and allowing the animal access to the rest of the experimental room. Following the removal of a single wall 9 place cells shifted field location (with a mean correlation between the two maps of -0.07), 7 cells became active, 7 became inactive (<1Hz), 2 showed no change (one cell had a correlation of 0.67 between the two maps, the other a value of 0.75) and 8 were not active in both of the trials (see Figure 15 for typical results).

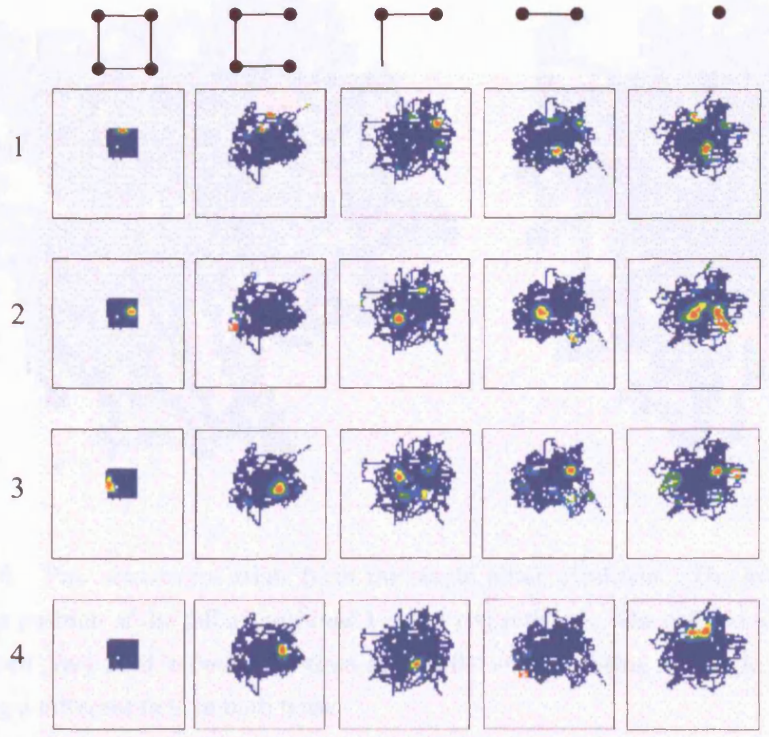


Figure 15. Representative responses of four simultaneously recorded place cells to the removal of boundaries. Top row shows the manipulation performed (closed box, 3 walled box etc). Cell numbers are on left.

4.3.4. Single-pillar analysis

The mean correlation coefficient for trials where the pillar was moved around the experimental room was 0.20 (see Figure 16 for an example of single pillar trials with low correlation scores and Figure 17 for a counterexample).

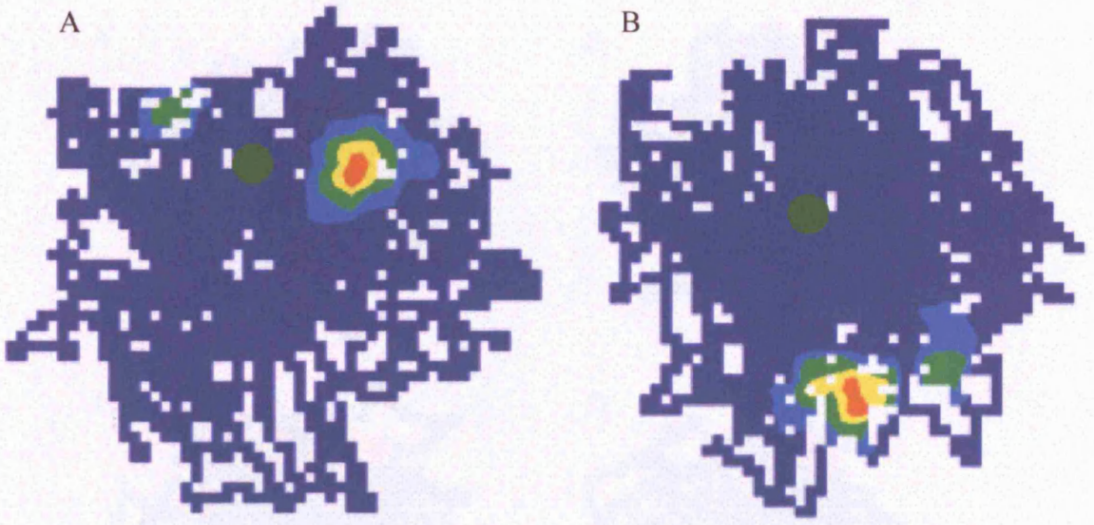
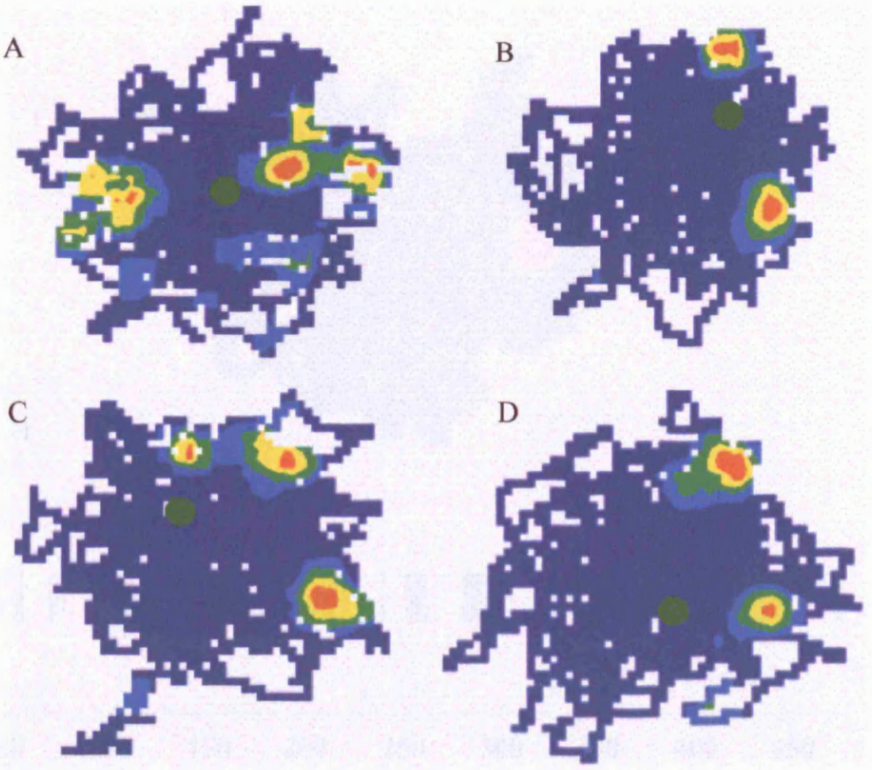


Figure 16. Two concurrent trials from the single pillar condition. The green circle shows the position of the pillar (positions 1 and 4 respectively). The cell has a coherent, well-formed place field in both conditions despite the only cue being the single pillar and exhibiting a different field in both trials.



E

Map	A	B	C	D
A	-	-0.1	-0.09	-0.13
B		-	0.64	0.59
C			-	0.68
D				-

Figure 17. A place cell recorded across four consecutive single pillar trials. The place cell develops a stable place field locked to the experimental room in the final three trials. The green circles depict the position of the pillar, white squares are unvisited pixels. The correlations between each of the maps are shown in E).

Examining cell activity across time in addition to space reveals that cells active in the single pillar condition with well defined place fields were active across the whole trial and not just in one concentrated burst (see Figure 18).

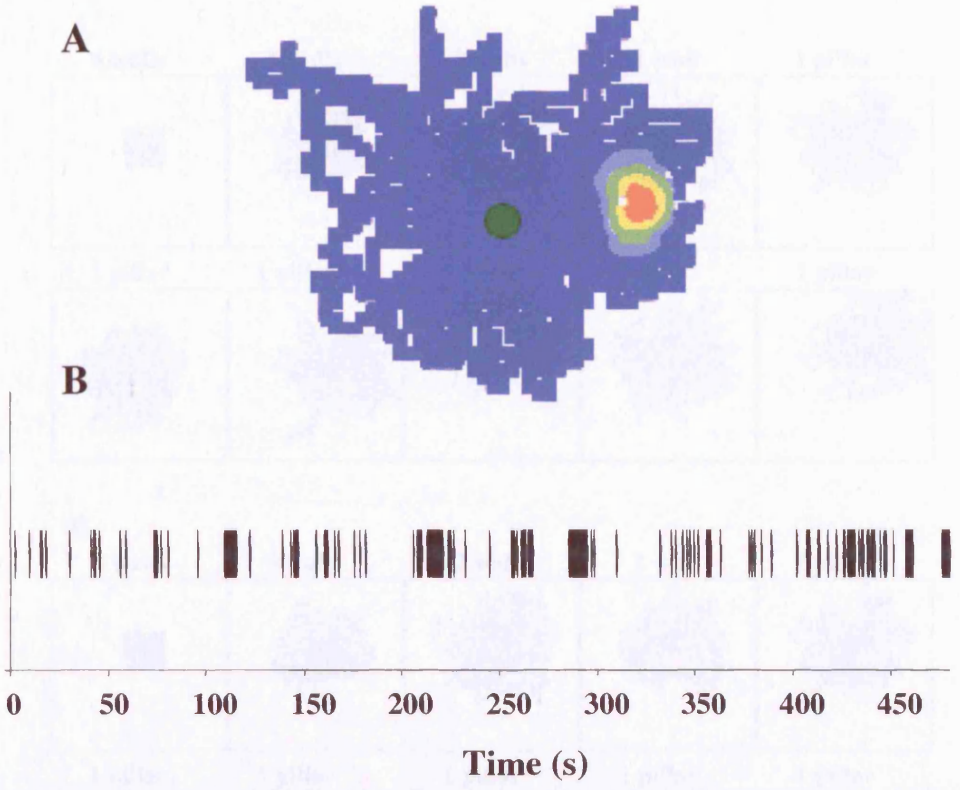


Figure 18. Place field and raster plot from a cell recorded in the single pillar condition. A) Place field is to the east of the pillar. B) Raster plot from a section of the trial showing the cell fires throughout and not in a single, localised burst.

4.3.5. Room frame

4.3.5.1. Whole room

The room frame/ whole room analysis represents the original, "untransformed" data set, and therefore is a baseline from which to compare the other types of analysis. Here the activity of cells is examined across all of the experimental room, both inside and outside the local box (see Figure 19).

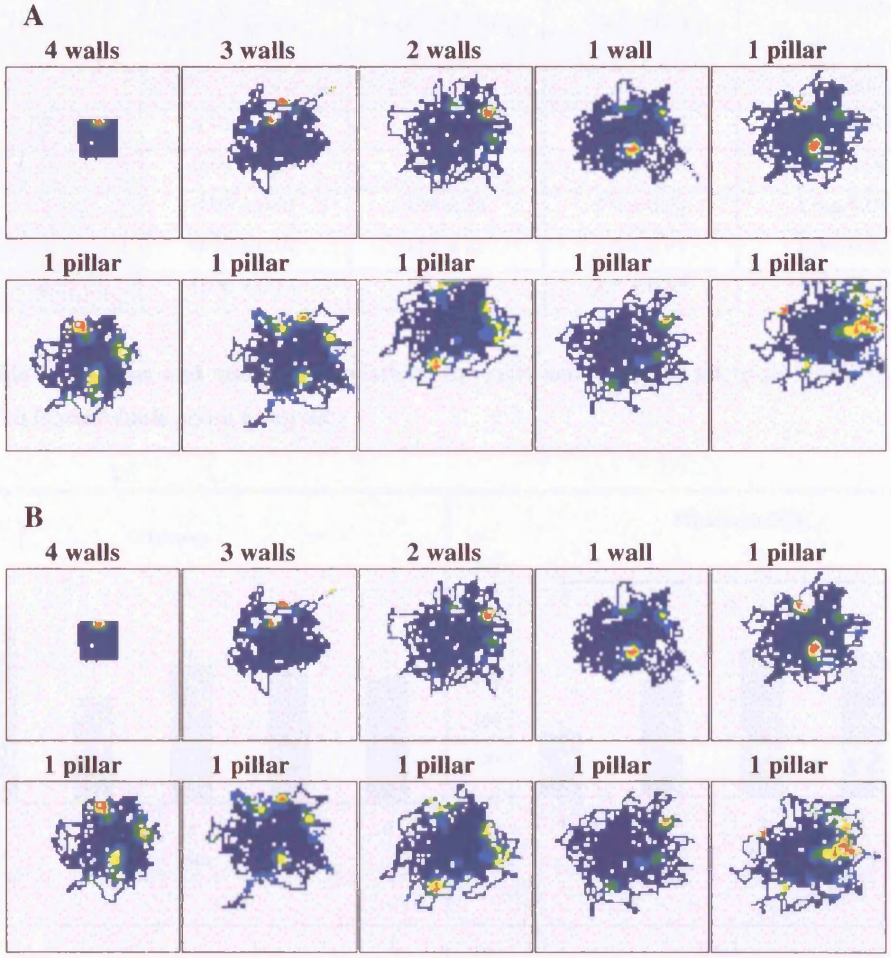


Figure 19. Place fields from cells in the all room assessment method. A) Fields from the Room frame/whole room analysis. B) Fields from the Box frame /whole room analysis. Note that the firing rate maps in the B have been realigned to the central box position (position 5) compared to the firing rate maps shown in A. Empty (white) areas indicate unvisited pixels.

Walls	Coherence	Pixels with firing	Rate (Hz)	Spatial Information Content (bits/ spike)
0	0.91 ± 0.02	227 ± 29	3.8 ± 0.55	0.99 ± 0.14
1	0.91 ± 0.01	186 ± 22	4.5 ± 0.98	1.2 ± 0.09
2	0.92 ± 0.01	194 ± 25	3.9 ± 0.78	1.0 ± 0.09
3	0.90 ± 0.02	152 ± 27	3.6 ± 0.94	1.3 ± 0.22
4	0.96 ± 0.01	83 ± 13	2.7 ± 0.65	1.4 ± 0.19

Table 1. Means and standard deviations for cells active across all trials (n=8) in the Room frame/whole room analysis.

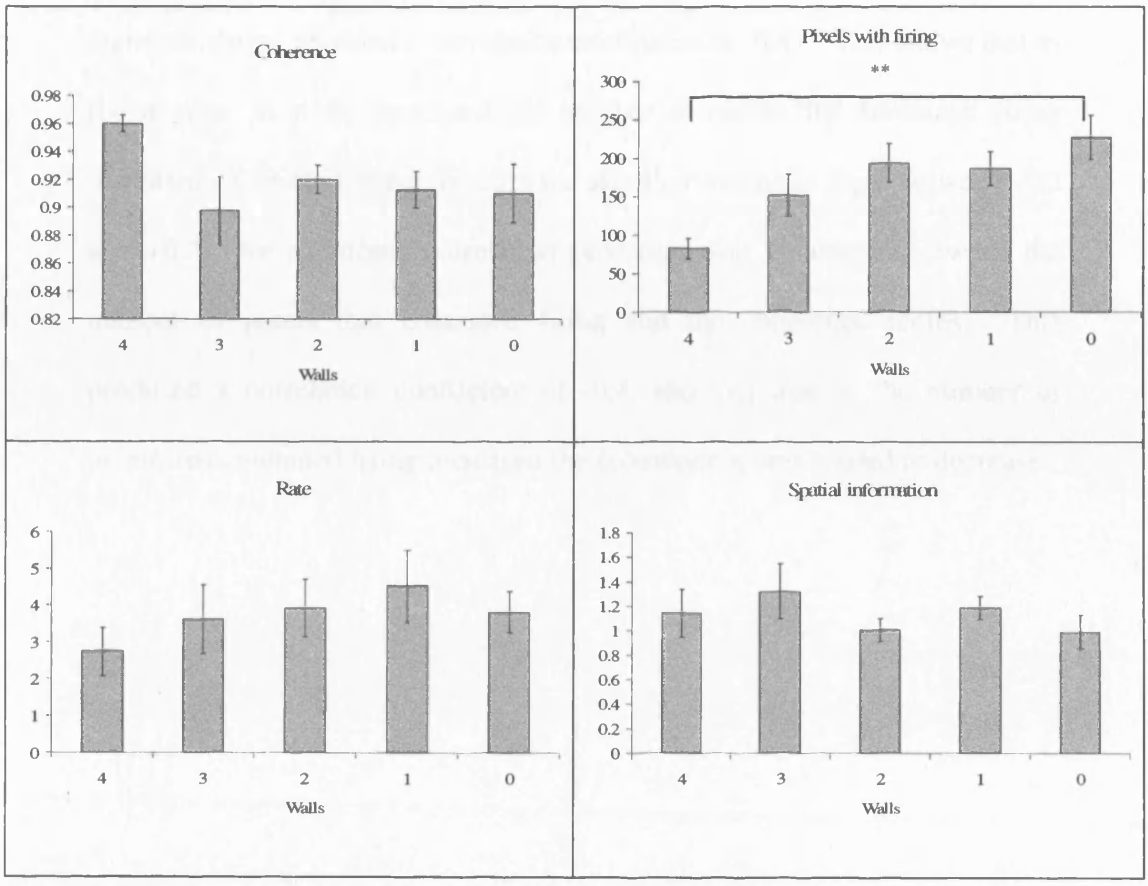


Figure 20. Firing Area, Coherence, Rate and Spatial Information content for cells across all trials in the Room frame/whole room assessment. Double asterisk indicates a significant difference < 0.01.

One-way repeated measures ANOVAs were used to assess differences across conditions in the Room frame/whole room assessment method. Only cells

active across all conditions were used in the analysis (n = 8) and, as there were multiple single pillar trials run, only the first single pillar trial in a session was included in the analysis. There were no significant differences for firing rate, coherence or spatial information content. However, there was a significant difference in the number of pixels that contained firing ($F_{(4,35)} = 5.22$, $p < 0.01$). Post hoc tests revealed a significant difference between the 4 walled condition and the single pillar condition. Furthermore, performing a correlation between the number of walls and the number of pixels that contained firing revealed a correlation coefficient of -0.57. This shows that as the number of walls decreased the number of pixels that contained firing increased. Correlation coefficients for all other measures were between -0.2 and +0.2. An additional correlation procedure was conducted between the number of pixels that contained firing and the coherence scores. This produced a correlation coefficient of -0.4, showing that as the number of pixels that contained firing increased the coherence scores tended to decrease.

4.3.5.2. Box only

The Room frame/box only assessment examined firing that only occurred in the local box and ignored firing outside the box. See Figure 21 for an example of the maps that were correlated using this procedure.

Walls	Coherence	Pixels with firing	Rate (Hz)	Spatial Information Content (bits/ spike)
0	0.91 ± 0.02	99 ± 17	3.4 ± 0.78	0.79 ± 0.2
1	0.88 ± 0.03	91 ± 15	3.9 ± 1.1	0.92 ± 0.2
2	0.91 ± 0.02	113 ± 14	2.5 ± 0.59	0.62 ± 0.1
3	0.91 ± 0.02	79 ± 9	2.5 ± 1.1	0.98 ± 0.15
4	0.95 ± 0.01	81 ± 13	2.6 ± 0.67	1.1 ± 0.21

Table 2. Means and standard deviations for cells in the Room frame/box only assessment method

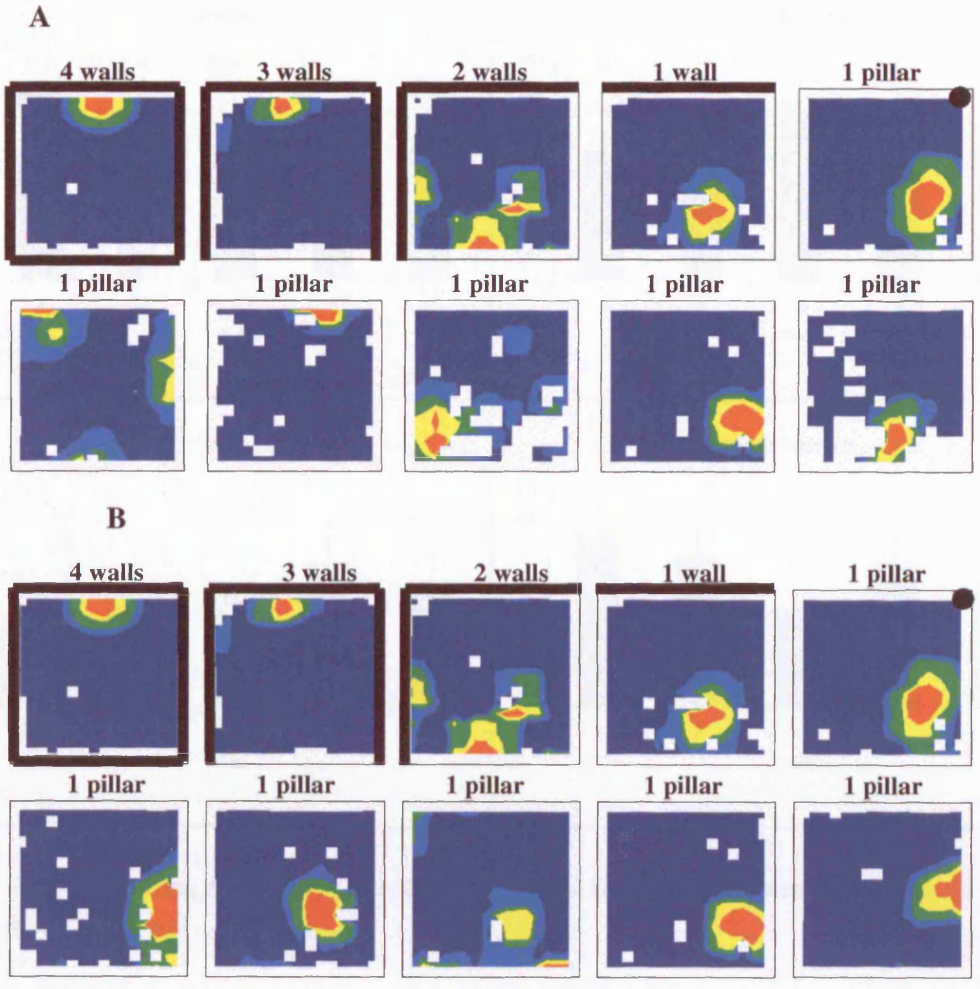


Figure 21. Place fields for a single cell analysed using the box only assessment method. A) Fields from the Room frame/box only analysis. B) Fields from the same cells using the Box frame/ box only analysis. Note that the top row of both A and B are identical as no movements of the box have yet occurred. The bottom two rows are different because the firing rate maps in B have been realigned to the central box position. Empty (white) areas indicate unvisited pixels. Solid black lines and circles indicate the positions of the remaining walls/ pillar for the first 5 trials only as the pillar was moved further away on the last five trials and are omitted for clarity.

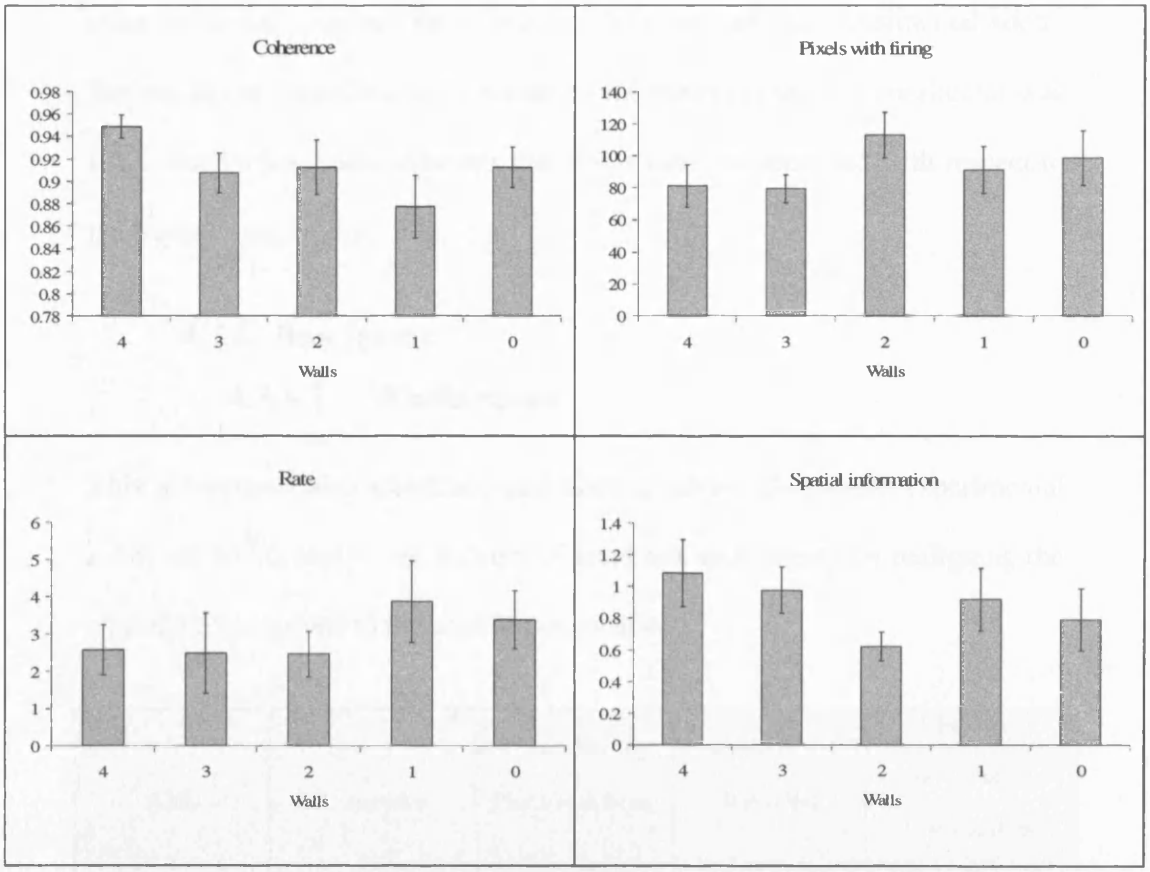


Figure 22. Firing Area, Coherence, Rate and Spatial Information content for cells in the Room frame/box only assessment.

Each of the four measures was assessed for differences between the different trial types (4 walls, 3 walls etc.). Only cells active in all conditions were entered into the analysis (n = 8). As there were multiple single pillar trials run only the first single pillar trial in a session was included in the analysis. A series of repeated measures ANOVAs revealed there were no differences between the conditions in terms of firing rate, firing area, coherence or spatial information.

In order to examine the effect of moving a single pillar to different locations in the experimental room, the mean for the correlations of all place field maps in trials where the pillar was moved was calculated. A high value would indicate

place cells that localised their fields with respect to the experimental room. For the Room frame/box only condition the mean correlation coefficient was 0.22. Such a low value indicates that fields were not localised with respect to the experimental room.

4.3.6. Box frame

4.3.6.1. Whole room

This assessment also examined cell activity across the whole experimental room but additionally took account of local box movements by realigning the shifted box locations to the central box location.

Walls	Coherence	Pixels with firing	Rate (Hz)	Spatial Information Content (bits/ spike)
0	0.81 ± 0.09	162 ± 57	3.4 ± 0.86	1.48 ± 0.35
1	0.75 ± 0.12	88 ± 31	3.2 ± 1.1	1.51 ± 0..34
2	0.77 ± 0.11	116 ± 41	3.3 ± 1.2	1.5 ± 0.35
3	0.80 ± 0.1	112 ± 40	2.8 ± 0.95	1.64 ± 0.39
4	0.65 ± 0.15	54 ± 19	1.41 ± 0.74	0.94 ± 0.31

Table 3. Means and standard errors for cells active across all trials for the Box frame/whole room analysis.

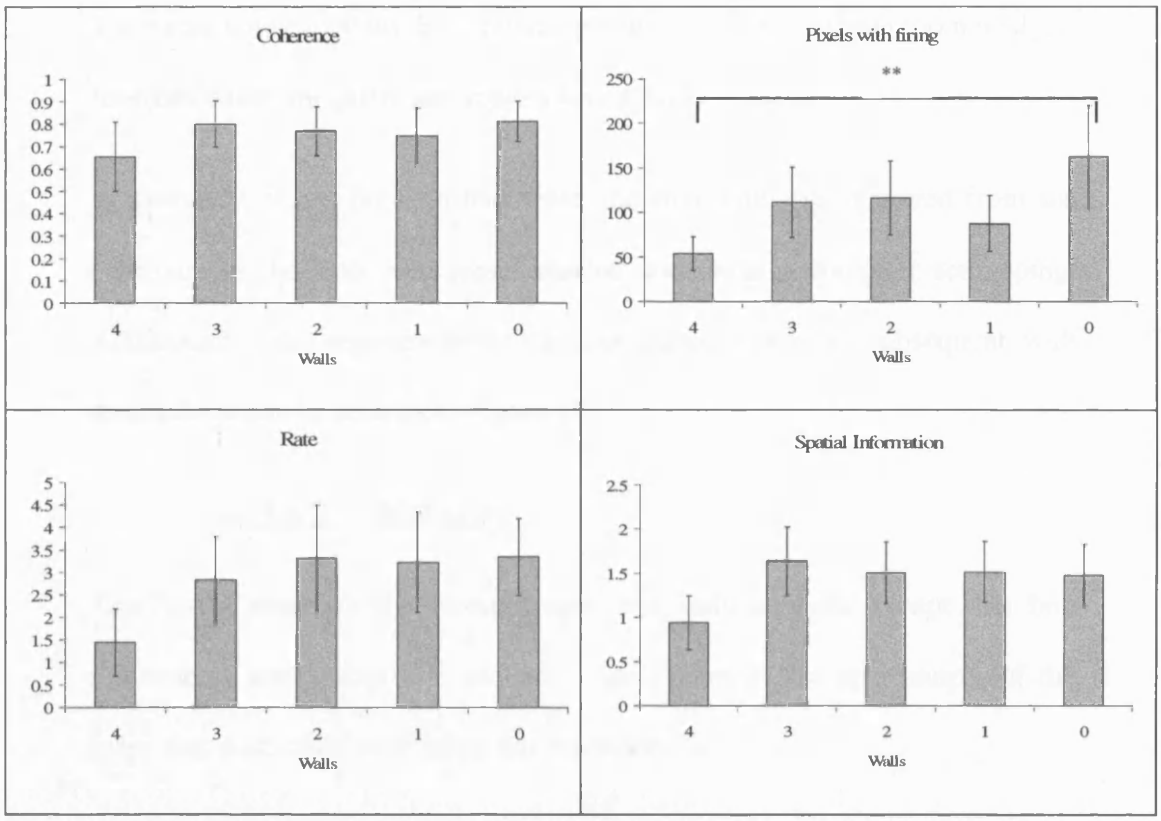


Figure 23. Firing Area, Coherence, Rate and Spatial Information content for cells in the Box frame/whole room assessment. Double asterisk indicates a significant difference < 0.01.

Differences between the various measures were investigated using a series of one-way repeated measures ANOVAs for the Box frame/whole room condition. Only cells that were active in all of the different box conditions were included in this analysis (n = 8). As there were multiple single pillar trials run only the first single pillar trial in a session was included in the analysis. There were no significant differences for spatial information, coherence or firing rate. However, there was a significant difference in the number of pixels that contained firing ($F_{(4, 35)} = 5.25$, $p < 0.01$). Post hoc tests revealed a significant difference between the 4 walled condition and the single pillar condition.

The mean within-condition correlation for the Box frame/whole room analysis for trials where the pillar was moved was -0.04.

In summary, it can be seen that when the first wall was removed from the environment the place cell representation underwent a complete remapping. Additionally, the representation was not stable following subsequent wall removals as can be seen from Figure 15.

4.3.6.2. Box only

This is the same as the Room frame/ box only analysis except that box movements were taken into account. See Figure 21 for an example of the maps that were correlated using this procedure.

Walls	Coherence	Firing area	Rate (Hz)	Spatial Information Content (bits/spike)
0	0.92 ± 0.02	89 ± 18	3.4 ± 0.78	0.81 ± 0.19
1	0.88 ± 0.03	91 ± 15	3.9 ± 1.1	0.92 ± 0.2
2	0.92 ± 0.03	117 ± 14	2.5 ± 0.59	0.62 ± 0.1
3	0.91 ± 0.02	86 ± 10	2.7 ± 1.1	0.92 ± 0.16
4	0.95 ± 0.01	83 ± 13	2.6 ± 0.67	1.1 ± 0.19

Table 4. Means and standard deviations for cells in the Box frame/box only condition.

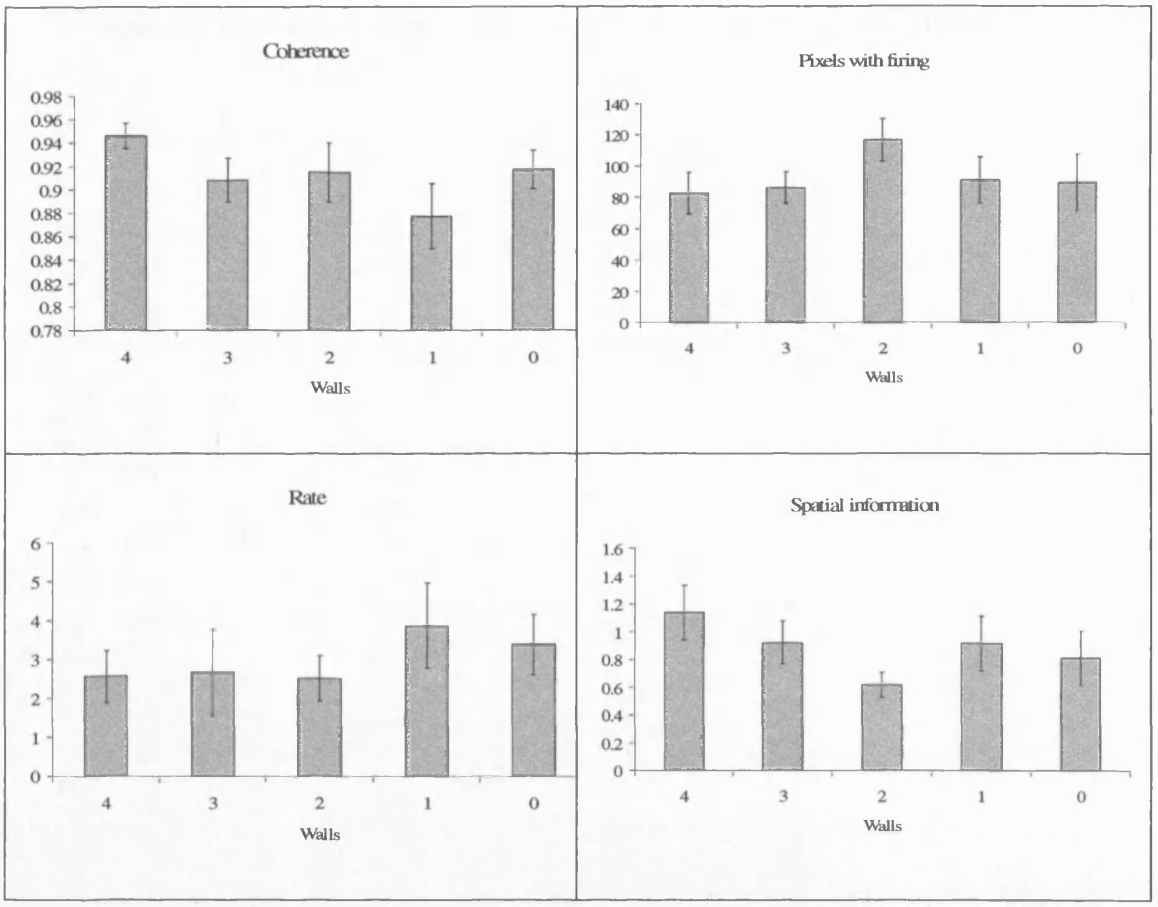


Figure 24. Firing Area, Coherence, Rate and Spatial Information content for cells in the Box frame/box only assessment.

Firing rate, firing area, coherence and spatial information content were each assessed using repeated measures ANOVA's (see Table 4 and Figure 24). Only cells active in all conditions were used (n = 8) and only the first single pillar trial. A series of repeated measures ANOVAs revealed there were no differences between the conditions in terms of firing rate, firing area or coherence. However, spatial information for cells in the Box frame/box only assessment did approach significance, ($F_{(4,35)} = 2.71$, $p = 0.051$) due to the low values in the 2 walls condition. The mean correlation coefficient for the trials where the pillar was moved to different locations in the experimental room was -0.02. This low value indicates that place fields in the single pillar

condition were not oriented with respect to a Box-based reference frame.

4.4. Discussion

The main finding from the current experiment is that following the removal of environmental boundaries place fields occupied larger portions of the experimental area. This effect can be seen in Figure 19 where there is a gradual increase in the area that contains firing as the number of boundaries removed increases. This was accompanied by a parallel decrease in the coherence of place fields, as shown by the negative correlation between the two measures (-0.4). The removal of barriers also resulted in place fields undergoing other dramatic changes in their firing patterns. This manifested in several ways. First, place fields exhibited a complete remapping when a single wall was completely removed from the four-walled environment. This can be seen in Figure 15 in the first and second columns. The first cell appears to be an exception and maintains a very similar field in the three-walled environment as the bounded environment. Cells two and three however shift their firing fields to novel locations in the environment. The second cell forms a field that is to the west of the west wall – notably this is outside the region described by the remaining boundaries. The third cell remaps to a novel location within the boundaries described by the box, and the final cell becomes active when the wall is removed. Such a change is indicative that a complete remapping has occurred.

Second, place field activity did not seem to be bound to the box region as might be expected given models of place field formation (Hartley et al., 2000;Lever et al., 2002a), a result seen with cell three described above. Third, place fields of the same cell were very poorly correlated across separate trials when the location of a single pillar was moved around the experimental room.

This indicates the pillar had poor control over place fields from trial to trial. Last and perhaps most interestingly, was that despite these dramatic changes to place fields they were still able to form in an unbounded environment, although this was not common (see Figure 17 for an example). That is, despite showing different fields from trial to trial a cell was still able to form "normal" place fields. This occurred even when the only stimulus remaining was a single pillar, indicating that boundaries per se are not necessary for place field formation. However they do appear necessary for stabilising fields across trials. This finding is contrary to predictions derived from modelling efforts aimed at explaining why place cells fire where they do (Hartley et al., 2000;O'Keefe and Burgess, 1996).

The type of remapping that place fields underwent when the first wall was removed was what was referred to earlier as a complete remapping; cells switched on or off, or shifted their preferred firing location. There was a very small minority of cells (<10%) that exhibited no change in their firing patterns. As will be seen in Experiment 3 such a complete remapping is possibly indicative that a contextual change has occurred. An extension to this idea, returned to later, is that geometric information can be fed back into the hippocampus as contextual information (see Anderson et al., 2004). This would result in a complete remapping occurring following a geometric alteration to an environment. Apart from the obviously missing wall, the removal of one boundary also results in the loss of two corners. It seems that the three-walled environment was treated (by the place cell representation at least) as completely different to the four-walled environment. This is evidenced by the low correlation scores between the bounded, four-walled

environment and the unbounded, three-walled environment (0.15). Although no quantitative measure was taken of the animals' behaviour it can be reported anecdotally that there was no immediately obvious change in behaviour following the removal of the first wall. As can be seen from Figure 14 animal's readily ventured outside the area bounded by the three remaining walls and seemed to return quickly upon encountering the loud white noise barrier. There were no other noticeable changes in the amount of rearing, freezing, defecation etc.

The different types of analyses were performed to see if place fields were responding to one of several possible reference frames (Redish, 2001;Rosenzweig et al., 2003). The two main reference frames that could have been used were one defined by the room itself and one defined by the local box environment. The analyses that examined place field activity that only occurred in the region defined by the box (the Room frame/box only and Box frame/box only analyses) revealed no significant differences between any of the measures employed. Part of the reason for this analysis was to examine the possibility there might have been residual firing occurring in the box region that could have been attenuated by out-box firing. It was thought possible that place cells may continue to maintain fields in the region defined by the remaining box walls when a wall (or more) was removed, and furthermore that this firing could be attenuated ("washed-out") by strong firing occurring outside this region. This could occur via a burst of strong firing happening in a poorly sampled location outside the box. This would result in an undue weighting of the firing rate map to that location. A problem such as this would be overcome by examining only cell activity that occurred within

the box environment (or the area previously described by the barriers). Therefore the box only analysis, as well as examining activity in the reference frame of the box circumvented this methodological issue. The results do not support the idea that cells did use the box as a reference frame and, as can be seen from Figure 21B, place fields did not maintain the same position in the box from trial to trial. Although it appears from this figure that the cell has a reasonably consistent field in the bottom right-hand corner of the box area, the field can be seen to move around between trials. Indeed this was the "best" example of a cell that displayed such a persistent field within the boundaries described by the box.

Further examination of the possibility that a burst of strong firing occurring in a poorly sampled location outside the box could have unduly weighted the firing rate maps is provided by the temporal analysis of place cell firing. The raster plot in Figure 18 shows that place cells fired across the entire trial and not in one concentrated burst. This shows that place cells fired not just on a single pass through the field but on repeated passes through it. Examination of the paths of the rats as they moved through the field also shows that the rats made repeated passes through a field. Animals did sample some areas of the environment poorly, particularly at the edges of the area they traversed – for an example, see Figure 17D; at the lower part of the area covered by the animal there is a low amount of firing. In this case no undue weighting to the rate map is apparent.

The analyses that examined activity across the whole environment (i.e. inside and outside the local box environment) revealed significant differences

between the numbers of walls in terms of the number of pixels that contained firing. There was also a trend for the coherence to be higher for the bounded, 4-walled environment than any of the unbounded conditions in all of the analyses except the Box frame/whole room analysis. Taken together with the negative correlation between pixels with firing and coherence, these results suggest that either the effect of removing a boundary was to increase the area over which a cell fired or that the presence of boundaries constrained the firing of place cells. These possibilities are not mutually exclusive. Inspection of the firing rate maps of cells in the unbounded conditions shows that fields did not resemble the "nice and neat" place fields seen in the bounded environment. It is also possible that the fields seen in the unbounded conditions reflect the upper limits on the size that place fields from CA1 can take. If this is the case then when recorded in a bounded environment, place cells are in some sense constrained by the presence of enclosing boundaries. This is further supported by findings from Muller & Kubie (1987a) and Burgess & O'Keefe (1996) that scaling up the size of an environment can similarly increase the size of the place fields (without causing a field-shift type remapping).

Other firing patterns such as the residual firing occurring in the box region could also have included the presence of "ghost" fields. By reducing the boundaries it was possible that long elongated firing fields reminiscent of what a putative BVC receptive field looks like would be seen (see Figure 9). This was never observed. The BVC model captures very well the appearance of place fields – they are commonly aligned with their long axis parallel to a boundary/barrier and have an Gaussian profile over the course of a trial. Even

when boundaries were removed from the environment and fields broke down and formed several subfields, such a Gaussian profile was still obvious (see Figure 15). Therefore in order to form coherent, individuated place fields it seems as though place cells need to be able to integrate inputs from many boundaries. This is best achieved in a fully bounded, enclosed environment, where they can integrate inputs from a full 360°.

The lack of stability seen in the reduced wall conditions is also supported by the place fields recorded in the presence of the single pillar. Here the correlations across all single pillar trials were very low. In Experiment 3, place fields that displayed no change in their firing parameters had correlation values of >0.7, whereas those that had undergone a complete remapping had values around 0.1-0.3. These lower values are typical of those seen in the single pillar trials in Experiment 1. Although caution should always be exercised when making comparisons of this type, the correlations on single pillar trials are very low and reflect unrelated place fields from trial to trial. This can be seen by looking at the single pillar trials in Figure 16 and Figure 17. The correlation values for the single pillar trials were low regardless of the analysis used (i.e. whether the whole room or just the box was considered, and if the maps were shifted to account for box translations or not)[8]. Overall this shows that the single pillar had very poor control over place fields, a

[8] A second type of analysis that could have been carried out would involve calculating the correlation between a place field rate map and rotated versions of itself. If the place cell had rotationally remapped then the position of the highest correlation would provide an indication of the amount the field had rotated. Such an analysis would also have to take into account the distance of the field from the pillar as this would need to remain constant for a rotational remapping to have been said to occur. This additional analysis was not performed here as fields in the single pillar condition were unstable from trial to trial and generally exhibited reduced coherence. In those rare cases where a well-formed place field was observed on consecutive single pillar trials the field(s) frequently changed their distance from the pillar (see Figure 16 for an example).

prediction made by the BVC model. Further support for the lack of stability is provided by the drop in coherence that occurs for all unbounded conditions. In addition to the coherence drop, the number of pixels that contained firing also increased. Despite this, place cells with a single, well-defined place field *were* active in single pillar trials (see Figure 16). As mentioned above, the occurrence of place fields in an unbounded environment is not well accounted for by current models of place field formation, except those based on path integration (Samsonovich and McNaughton, 1997). An additional possibility that would explain place field formation in an unbounded environment is that the place cell is firing in a temporally restricted manner as opposed to a spatially restricted one. However as can be seen from the example shown in Figure 18, cells were active throughout the trial.

The correlations of place cell activity in the single pillar trials averaged data over trials and so ignored any stability that may have occurred on a single trial. Indeed, it was occasionally possible to observe a reasonable degree of place field stability across several single pillar trials (see Figure 17). This occurred despite the pillar being moved from trial to trial and suggests that some form of spatial information *is* being retrieved on these trials. Because of the poverty of spatial information provided by the denuded environment is not possible to rule out that cells were anchored by local cues in the environment despite the extreme care that was taken to avoid this happening. At the start of every trial the transport box (containing the rat) was moved to the same position (just South of the North wall of the local box environment), the rat was removed, placed down "inside" the local box environment and the trial started. Because of the constant start position that was employed it remains a

possibility that stable place fields in these trials formed due to the rat remaining oriented via path integration. Although extremely unlikely it is possible that at the end of the trial, when the animal is picked up, it takes a "fix" on its current position and continues to update its position whilst inside the transport box. When it is removed from the box and placed down again the animal could still have had an accurate estimation of location (the transport box was not moved around in the inter-trial interval). Another possibility that could explain temporary stability is that the sound barrier served as a virtual barrier that functioned to localise place fields in the absence of extended surfaces. This cannot be totally ruled out but seems unlikely as fields were never seen that treated the white noise barrier as a barrier in the same sense as a wall. That is, place fields were never observed that fired with their long axis aligned to the sound barrier as they do next to physical barriers such as the walls of the environment used here.

Although place cells can form transient fields from trial to trial, in order to form stable fields that reliably occur in the same location information derived from boundaries appears important. As the BVC model stands there is no account of the influence of plasticity, a process that is presumably important for stabilising place fields over long periods (i.e. across trials/ sessions and days; see for e.g. (Agnihotri et al., 2004;Kentros et al., 1998). A potential mechanism that would explain the changing place fields from trial to trial can however be postulated based on some of the assumptions of the boundary vector cell model. The BVC model posits that initially the boundary inputs to place cells are randomly selected. It is possible in the current experiment that the lack of consistent boundary information from trial to trial means that

random sets of boundary inputs are selected anew at the start of every trial. This would result in different place fields being instantiated at each trial and could certainly account for the difference in place cell activity seen between the four and three walled trials. However, as mentioned above, when viewed through the mechanism elaborated in the General Introduction for remapping, the type of remapping observed between these two trials is complex and complete. Therefore this indicates that a contextual change has occurred reflected in the activity of place cells. A simplified mechanism to explain this can be postulated as follows. When one of the walls is removed this effectively silences the input to a place cell from a boundary vector cell attached to the removed wall. In order to maintain the level of drive to the cell other boundary vector inputs (along with the other previously active ones) become predominant. This therefore allows other boundary vector inputs to gain influence over the cell and cause it reach threshold. Such an altered distribution of boundary vector inputs will result in a place cell becoming active or inactive or shifting its firing field. All of these patterns of responding were seen in equal amounts in the current experiment, with very few cells displaying no change.

The contextual change in activity of the place cells may be due to the fact that the only time the animal is subjected to a loud noise is when one of the walls is removed (and it encounters the sound barrier). Therefore the change in context could be related to stress upon encountering the sound barrier. Place cell remapping has been observed during fear conditioning paradigms (Moita et al., 2004). However in such experiments the fear response is measured in terms of the percentage of time the animal spends freezing. Although not

explicitly measured in the current experiment, there was no notable change in rats' behaviour apart from a rapid return to the "silent" zone (no changes were observed in the amount of time the animal spent locomoting, defecating or urinating). Therefore it seems as though the effect of the sound barrier was not enough, in itself, to induce a potent fear response. Given this it seems unlikely that the remapping observed was a result of a change in context due to stress.

Place field stability may also be correlated with the task the animal is asked to perform, or the amount of attention it is required to pay to spatial cues or context in order to perform a task (Kentros et al., 2004). The current experiment did not explicitly require animals to use spatial information to "solve" a task. Indeed there was no task to perform above chasing after food thrown on the floor, quite possibly a more ecologically valid behaviour than pressing a lever or being electrocuted. However it is possible that if the experiment contained a task that required animals to pay "attention" to and use the spatial information derived from the walls and/or pillars, then place field stability and coherence may have increased in the unbounded conditions.

The results from Experiment 1 can be interpreted in terms of the BVC model in the following fashion. As boundaries/barriers are removed from the environment the place cells are increasingly deprived of BVC input. Because BVCs respond at a preferred direction from a boundary, a single boundary can only provide 180° input at maximum, whilst complete input is only provided by a completely enclosed environment. In theory the directional cue included on the distal wall in Experiment 1 combined with a single local cue (e.g. the single pillar) provides enough information to uniquely identify locations

within an environment. In practice it appears that this is not enough to accurately localise *consistent* (i.e. stable from trial to trial) place field activity. Despite the lack of overall session stability these two cues may provide enough spatial information to form place fields on a trial-by-trial basis, as evinced by place fields such as those seen in Figure 16, and the low coherence and high numbers of pixels that contained firing in the single pillar conditions. The existence of place fields when there is no extended surface for boundary vector cells to respond to suggests that the model is not complete and would require extension to incorporate these findings.

As mentioned above it is also possible to interpret the results from Experiment 1 in terms of the effects of context on place field activity. That is, the spatial alterations made to the environment may have been interpreted by the place cell ensemble as a contextual rather than spatial change. For example, Lever et al (2002) found remapping between a square and a circular environment. This remapping developed from representations that were initially highly similar in the two different enclosures. With time the representations came to diverge until they could no longer be said to be overlapping. Such a change was stable over extended periods of time. It is conceivable that the remapping seen was a result of a contextual change disguised as a spatial one. As mentioned in the General Introduction a context constitutes almost everything in the environment, including spatial stimuli.

Following this reasoning through results in the conclusion that even features that up to now have been called spatial (such as the boundaries of an environment) can, under certain circumstances, constitute contextual elements.

For example, in the Lever et al (2002) experiment it is possible that the presence or absence of corners (in the square and circle) functioned as contextual rather than spatial elements cues despite this being at odds with the definition of geometric and non-geometric sources of information given by Gallistel (1990). In Experiment 1 the removal of a wall could also constitute a contextual change to the environment. After the wall is removed the rest of the experimental room is available to the animal to explore. The rest of the room certainly constitutes a new environment (at least on the first exposure), albeit one that the animal had limited visual access to (the walls of the local box being transparent). The type of remapping exhibited by the cells when the first wall was removed was certainly reminiscent of a complex remapping: 14 cells switched on or off and the remainder of the active cells in the first two trials (9) shifted field location. If place fields were strongly determined by the boundaries of the local box environment then at least some of the fields (maybe those furthest from the removed wall) should be resistant to the wall removal. Admittedly, the removal of one whole boundary is a somewhat dramatic and slightly unusual change to make to an environment. On the human scale such an alteration can signify either the mundane (opening a door) or the disastrous (collapse of the roof supported by the wall).

The general breakdown in the specificity of location-specific firing seen in Experiment 1 was investigated in further detail in Experiment 2. Here, smaller, less dramatic changes were made to the environment. Instead of completely removing one whole boundary at a time, a single wall was detached and moved away from rest of the box. It was hoped that such a manipulation would result in less drastic alterations in place field firing

occurring. Such manipulations would also help circumvent the possibility that wall removal was considered a contextual change by the place cell system. One of the conclusions from Experiment 1 is that place cells require input from several sources in order to be stable across trials. The lack of stability seen in Experiment 1 made it difficult to arrive at conclusions about what was occurring across trials other than a complete remapping. Therefore maintaining the 360° input to place cells as much as possible within a deconstructed environment was one of the extensions derived from Experiment 1. It was hoped that much greater stability across trials would be seen with this manipulation. This would also be a much more explicit test of the BVC model as it would maintain BVC input to place cells whilst at the same time allowing manipulation of the individual vectors that impinge upon a place cell.

5. Experiment 2

5.1. Introduction

This experiment was an extension to the conclusions and findings that resulted from Experiment 1. The environment used in Experiment 2 was very similar to that in Experiment 1. However, as discussed in the conclusion to Experiment 1, less dramatic changes were made to the environment used here. One of the conclusions from the previous experiment was that in order to form stable fields across trials, place cells need to be able to integrate input from several different boundaries. To this end a decomposed environment similar, but different from, that used in Experiment 1 was designed so that coherent input to place cells was maintained from as close to 360° as was practically possible whilst still decomposing the boundary vector input to place cells. The manipulations conducted were also simpler. Instead of sequentially removing boundaries from the environment only two types of trial were run; one in a fully enclosed environment and a second with a single wall moved away from the rest of the box. These two trials were then repeated many times. Consistently repeating the same trials would also provide a higher degree of stability to the environment than in Experiment 1. As discussed in the conclusion to Experiment 1 it is possible that each subsequent trial was treated as a wholly novel context. This would explain the pattern of complete remapping observed (a point returned to in greater detail with Experiment 3).

These manipulations would also allow an examination of the connection between a place cell and its BVC inputs in terms of plasticity. Repeatedly exposing the animal (and the place cells) to the same two environments would

hopefully permit learning-induced changes to occur amongst the BVC-to-place cell connections. One of the potential outcomes of moving a wall away from the rest of the environment predicted by the BVC model would be the appearance of "split" fields. In the enclosed, bounded environment it was expected that "normal" looking place fields would be observed. Moving one of the boundaries away from the rest of the box would have the result of separating out the BVC input for a given cell thus "pulling" apart the individual Gaussian tuning curves that provide input to that cell. This would result in a cell that had something resembling a dumb-bell shaped, dual field, a result seen with O'Keefe & Burgess (1996). The role of plasticity in these manipulations may be, over the course of several trials, to "favour" one field over the other, such that one field becomes dominant and the other attenuated. However such a finding was never seen in the previous experiment. To reiterate, this could have been because each manipulation was viewed as a wholly novel context by the place cell system.

Because the manipulations to the environment were less severe in Experiment 2, a modification was also made to the sound barrier that prevented animals from accessing the room boundaries. Now, as well as the temporal delay to the initiation of the white noise barrier, a spatial change was also introduced. Following activation of the white noise and subsequent return to the quiet zone the radius of the barrier was reset (within predefined limits) so that it was no longer in the same place as it was initially. This would additionally serve to attenuate the possibility of the barrier serving as a localising source of information.

Initially a single bounded trial was run. Then a single boundary was moved away from the other boundaries a sufficient distance to allow the rat access to the rest of the experimental room (roughly 15cm). The same wall was always moved away from the other boundaries in all trials (except one). The following trials were repetitions of these two trials. Several outcomes could have occurred. First, and most straightforward, results could have concurred with the O'Keefe & Burgess finding; place fields could maintain a fixed relationship relative to the walls nearest them. Similar results could also have been seen as with Experiment 1, inasmuch that moving a wall away from the local box could have led to a breakdown in place field activity (lower coherence and so on). If this happened then presumably this would have something to do with the fact that animals had access to the rest of the room after the box was opened out. This would implicate an experience-dependent alteration to a previously active representation. It is also possible that the experience the cells underwent (repeated exposures to open and closed configurations of the box) would have some impact on their subsequent responses to the environments. It was also possible that in addition to the split fields mentioned above that "ghost" fields could have occurred, a possibility not seen in Experiment 1 (see Experiment 1 Discussion). This would perhaps be a more likely finding in the current setup as place cells would now have as close as was possible to 360° input from the surrounding boundaries.

It was found that the results were largely consistent with the BVC model, although the field doubling as seen with O'Keefe & Burgess was not reliably seen. Over the course of several trials some place cells were seen to develop new place fields where before there had been none. This effect was very

similar to that seen in Lever at al., (2002). Thus the changes made to the environment in the current experiment may be more dramatic than the gradual changes made with Lever et al., (where this form of remapping took place over days and weeks) and yet less severe than the changes made in Experiment 1. Such findings suggest the involvement of plastic changes occurring to the inputs arriving at the place cells and furthermore propose a modification to the BVC model should occur.

5.2. Methods

5.2.1. Subjects

The data from 3 adult male Lister Hooded rats (350 – 420g) was used in this experiment. Handling, feeding and photo-periods were as described in the General Methods.

5.2.2. Surgery

All cells were recorded from CA1 – see the General Methods for details of surgery.

5.2.3. Recording Environments

5.2.3.1. Screening

The screening environment used was the same as that described in Experiment 1.

5.2.3.2. Main recording room

This was the same as the recording environment described in Experiment 1 with some small modifications. The 5W light that was present in the pilot part of Experiment 1 was no longer present. The white noise sound barrier was as described in Experiment 1 with some additional modifications to ensure that the boundary was not serving as a stable spatial cue. In order to further prevent this, the radius of the barrier was reset following each deactivation of the sound barrier. Following re-entry to the quiet zone, the radius of the imaginary circle would reset to a random value chosen from a range that was a predefined distance less than the radius of the loud zone and greater than a lower limit. The same process applied to the louder sound zone when the

animal re-entered the moderate white noise area; the lower limit here was a predefined distance from the moderate white noise area.

5.2.3.3. Local environment

This was the same as the local box environment used in Experiment 1 with an additional modification. In order to provide a localising cue, a sheet of cardboard of dimensions 64x65cm was painted with glow in the dark paint. This was made by mixing 40% (by volume) Ultra Blue "Glow-in-the-dark" phosphorescent paint (Glow Inc., MD, USA) with Ronseal polyurethane matt finish paint (Ronseal, Sheffield, UK). The sheet of painted cardboard was attached to the North wall of the local environment by transparent tape at the top and bottom. The fluorescent side of the cardboard sheet faced into the local box environment; the reverse side was untreated.

5.2.4. Recording protocol

5.2.4.1. Trial structure

A standard trial in a bounded environment was five minutes long. In a bounded trial all four walls were connected to form a complete box with no gaps to allow the rat access to the rest of the experimental room. This type of trial was always the first run in a session. The box position (with the exception of one wall, see below) was always in the same absolute location in the experimental room (position 5). Following the initial, bounded trial a second, unbounded trial was run. Here, one wall was detached with its two associated pillars and moved away from the rest of the box by a distance of ~15cm. The same wall was always moved away from the other boundaries in all trials (with one exception). These two trial types then alternated until the

end of the session. Typically 9 trials were run, starting and finishing on a bounded trial.

5.2.5. Handling details

See Experiment 1.

5.2.6. Single-Unit recording

See General Methods for details of single-unit recording.

5.2.7. Place field measures

The same criteria used for Experiment 1 and described in the General Methods were applied for including a cell in the analysis for the current experiment. If any trial had a firing rate < 1Hz then it was discarded (although see below for an exception). The same measures used in Experiment 1 were used to assess cells in the current experiment, namely,

i. Coherence

ii. Spatial information content

iii. Firing rate

iv. Place field size (expressed as a ratio of the total area covered by the animal during a trial)

v. The total number of pixels visited by an animal during a trial

vi. The number of spikes fired by the cell and

vii. The number of pixels in which a cell fired.

These measures were used in two different types of analysis. The first looked at firing that occurred only whilst the rat was in the local box and ignored all other firing. This was called the box only analysis. The other type of analysis looked at firing that occurred throughout the whole environment. This was called the all room analysis.

In order to investigate any differences between the bounded and unbounded conditions in terms of the measures described above a series of paired t-tests were carried out. In order to carry out these tests, pairs of trials were selected where a cell was active in both conditions. All of the cells included in the analysis were active in at least one bounded and one unbounded trial (see below).

As mentioned above trials with a firing rate < 1Hz were discarded from the analysis as a cell was considered to be inactive below this rate. However, some of the cells in the current experiment displayed consistently low firing rates in the bounded condition and a highly similar firing field in the unbounded condition but with a much higher firing rate (a phenomenon similar, although not identical, to the "rate remapping" observed below in Experiment 3 and examined in greater detail there). For these cells, correlations between maps in the bounded and unbounded conditions and within maps in the same condition (called between condition correlations and within condition correlations respectively, as with Experiment 3) were examined as well as their trial-by-trial firing rate.

5.3. Results

A total of 11 cells were recorded from 3 rats (n = 4, n = 5, n = 2) that met the criteria for acceptance as place cells described in the General Methods.

5.3.1. Histology

Electrodes in all rats were confirmed to have been placed in region CA1 of the hippocampus. This was additionally confirmed by electrophysiological criteria (see General Methods).

5.3.2. All room analysis

Initially, place cell activity across the whole experimental room was examined. In the Bounded box conditions the place fields were consistent and stable across trials (see Figure 29, Figure 31 and Figure 33). For the Unbounded conditions there were differences in the pattern of cell responses (see the section Cell Classification). When the local box environment was in an Unbounded configuration the path of the animal was restricted by a varying sound barrier (see Experiment 1 & 2 Methods). An example of a typical path in an Unbounded trial can be seen below in Figure 25.

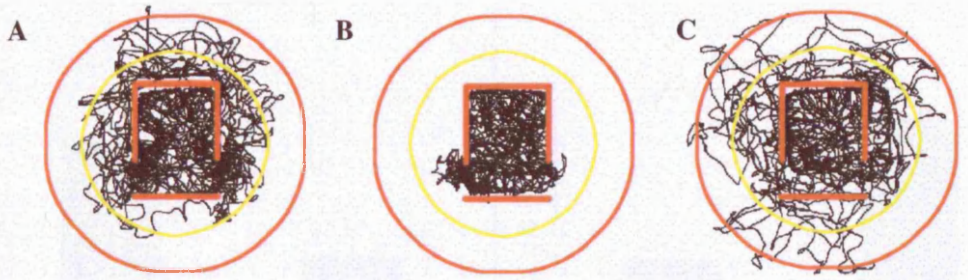


Figure 25. Typical paths for the three animals used in the current experiment. The local box environment is shown in the middle as straight red lines. The innermost extent the variable sound barrier could take is indicated with the yellow circle, the outermost extent by the red circle.

As can be seen from Figure 25 one of the animals was reluctant to leave the local box environment. The other two animals made occasional forays into the sound zone, but spent the majority of the time in the "silent" zone. There were no systematic differences in terms of the responses made by the cells recorded from each animal.

Four measures were used to assess differences in the Bounded and Unbounded conditions; coherence, firing area, firing rate and spatial information content. These are summarised in Table 5 and presented graphically in Figure 26.

Condition	Coherence	Pixels with firing	Rate (Hz)	Spatial Information Content (bits/spike)
Bounded	0.962 ± 0.021	174 ± 2	5.08 ± 4.12	1.66 ± 0.80
Unbounded	0.956 ± 0.039	464 ± 8	5.04 ± 2.97	1.88 ± 0.90

Table 5. Means and standard deviations for cells in the all room analysis

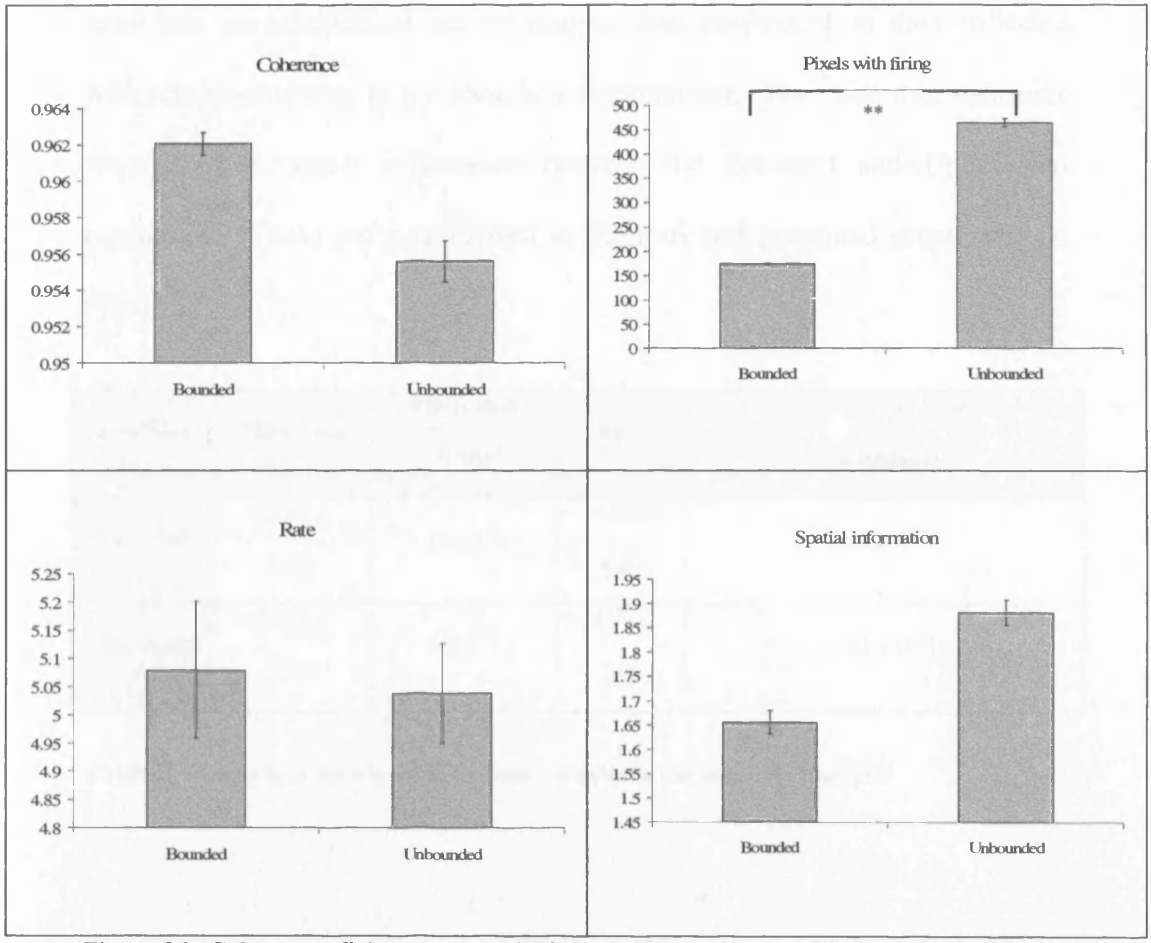


Figure 26. Coherence, firing area, spatial information content and firing rate data for cells in the room only analysis.

A series of paired t-tests were performed on each of the four measures shown in Figure 26 with configuration of the local box environment as the independent variable. There was no significant difference between the bounded and unbounded environments in terms of coherence, spatial information content or rate. There was a significant difference between the bounded and unbounded conditions for the pixels that contained firing ($t_{(33)}$ = 6.07, $p < 0.001$).

5.3.3. Box only analysis

Because there was no way to ensure sufficient spatial sampling outside the

local box environment, a second analysis was performed on data collected within the boundaries of the local box environment. The same four measures were used to assess differences between the Bounded and Unbounded conditions. These are summarised in Table 6 and presented graphically in Figure 27.

Condition	Coherence	Pixels with firing	Rate	Spatial information content (bits/spike)
Bounded	0.959 ± 0.031	184 ± 2	4.96 ± 4.20	1.50 ± 0.72
Unbounded	0.963 ± 0.039	309 ± 7	4.90 ± 3.08	1.41 ± 0.71

Table 6. Means and standard deviations for cells in the box only analysis

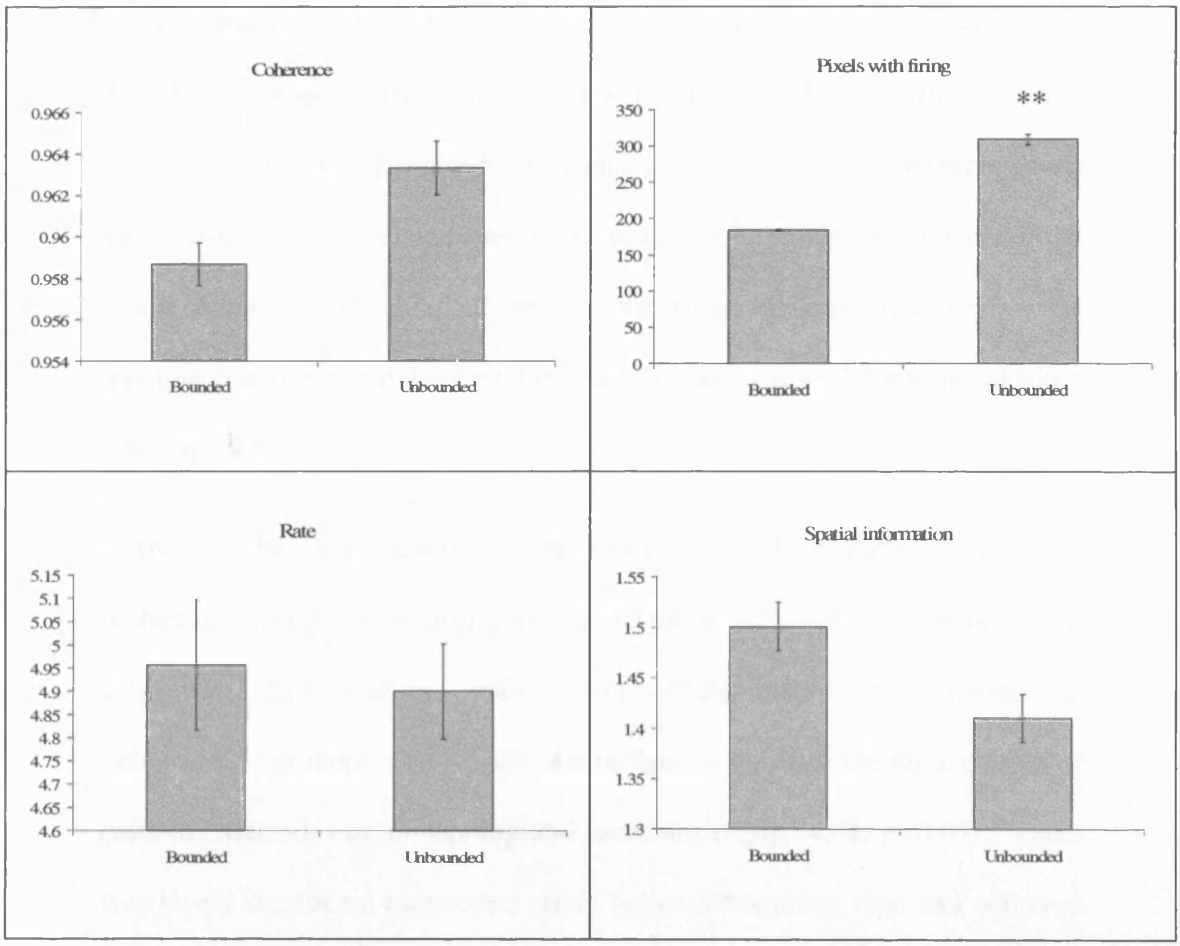


Figure 27. Coherence, firing area, rate and spatial information content for cells in the box only analysis

A series of paired t-tests were performed on these measures for the box only analysis. There were no significant differences for coherence, firing area, rate or spatial information. There was a significant difference between the bounded and unbounded box conditions for the total number of pixels that contained firing ($t_{(29)} = 3.06$, $p < 0.01$).

5.3.4. Cell classification

The room only and box only analyses examined population properties and therefore ignored individual responses of cells. Although there were only 11 cells recorded in this experiment, it was possible to divide cells into three

separate categories based on the response to shifting a wall out (see Figure 28). These categories were i) cells that were unaffected by the wall movement (n=4), ii) those that displayed a rate change with the wall movement (n=4) and, iii) those that became messy or unstable in the Unbounded condition whilst remaining stable/ consistent in the Bounded condition (n=3). As mentioned above, there were no differences between animals in terms of place cell responses.

A series of two-way ANOVAs were run on each of the different measures (coherence, pixels with firing, rate and spatial information content). For coherence, there was no main effect of boundary-type (bounded vs. unbounded) but there was a significant difference between the three classes of cells (unaffected, rate remapping and unstable) ($F_{(2,60)}$=4.61, $p<0.05$). There was also a significant interaction effect between boundary type and cell type ($F(2,60)=8.39$, $P<0.01$). Post-hoc tests revealed that this difference was between the unaffected and unstable groups.

The two-way ANOVA for pixels with firing with boundary type and cell type as factors showed a significant main effect of boundary type ($F(1,60)=95.4$, $p<0.001$) and cell type ($F(2,60)=38.2$, $p<0.001$) and a significant interaction ($F(2,60)=19.7$, $p<0.001$). Post-hoc tests revealed that this difference was between the unaffected and unstable groups and the rate remapping and unstable group.

A two-way ANOVA for rate showed no significant differences for either boundary type or cell type although the effect for cell type was almost significant ($F(2,60)=3.0$, $p=0.058$).

The two-way ANOVA for spatial information revealed an effect for cell type ($F(2,60)=17.5$, $p<0.001$). Post hoc tests showed this difference lay between the unaffected and unstable groups.

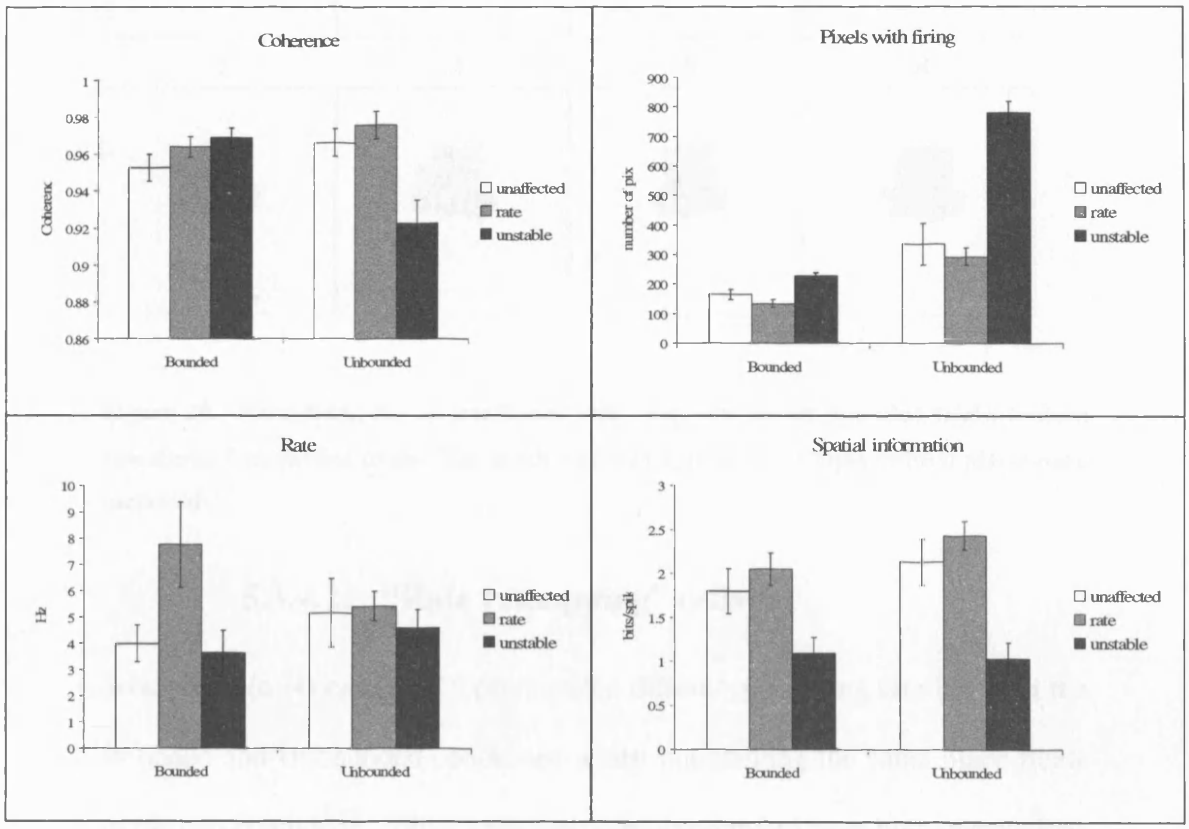


Figure 28. Coherence, pixels with firing, rate and spatial information measures for the three different types of cell response seen.

5.3.4.1. Unaffected cells

Some cells were unaffected by movements of a single wall (n=4). These had high correlation coefficients for the within and between condition correlations and also showed no significant differences in terms of firing rate, coherence, spatial information content or firing area (see Figure 28 and Figure 29). Most of the cells in the unaffected group had fields that were distant from the wall being manipulated (although no explicit measure of this was made).

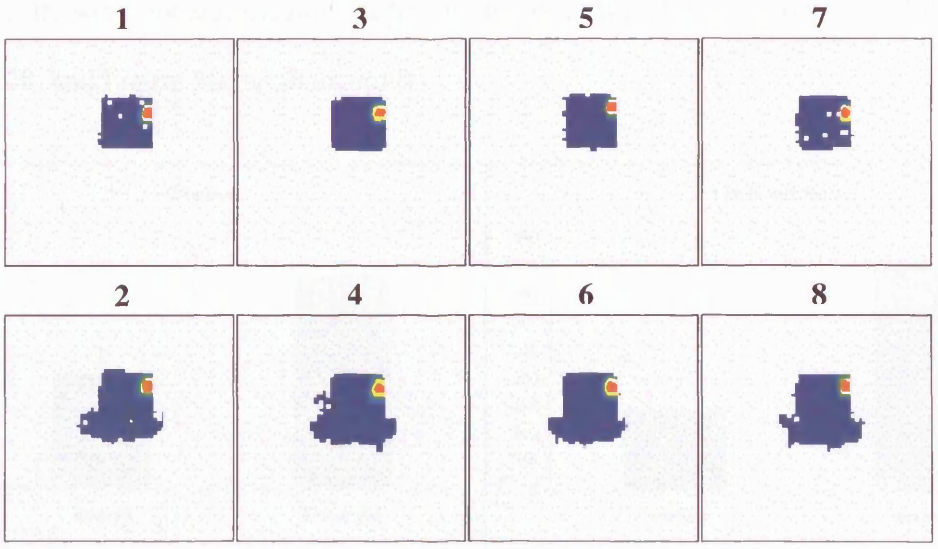


Figure 29. Place fields for an unaffected cell. Top row shows Bounded trials, bottom row shows Unbounded trials. The south wall was moved out. Empty (white) pixels were unvisited.

5.3.4.2. "Rate remapping" cells

Some cells (n=4) exhibited a pronounced difference in firing rate between the Bounded and Unbounded conditions whilst maintaining the same place fields in the two conditions. These cells would be expected to have high correlations for all conditions (within condition correlations i.e. bounded vs. bounded, = 0.86 and between condition correlations of 0.43). Figure 31 below shows the place fields from a cell that responded in such a fashion. All cells in this group had higher firing rates in the unbounded condition compared to the unbounded condition. A t-test comparing rate remapping cells in the bounded vs. unbounded conditions revealed a significant difference ($t(10)=2.8$, $p<0.01$). This group of cells were also significantly different in terms of the number of pixels that contained firing ($t(10)=8.68$, $p<0.001$). This may be due to the enlarged firing fields exhibited by cells firing at a higher rate. This group of

cells were not significantly different on any of the other measures (see Figure 28, and Figure 30 for an example).

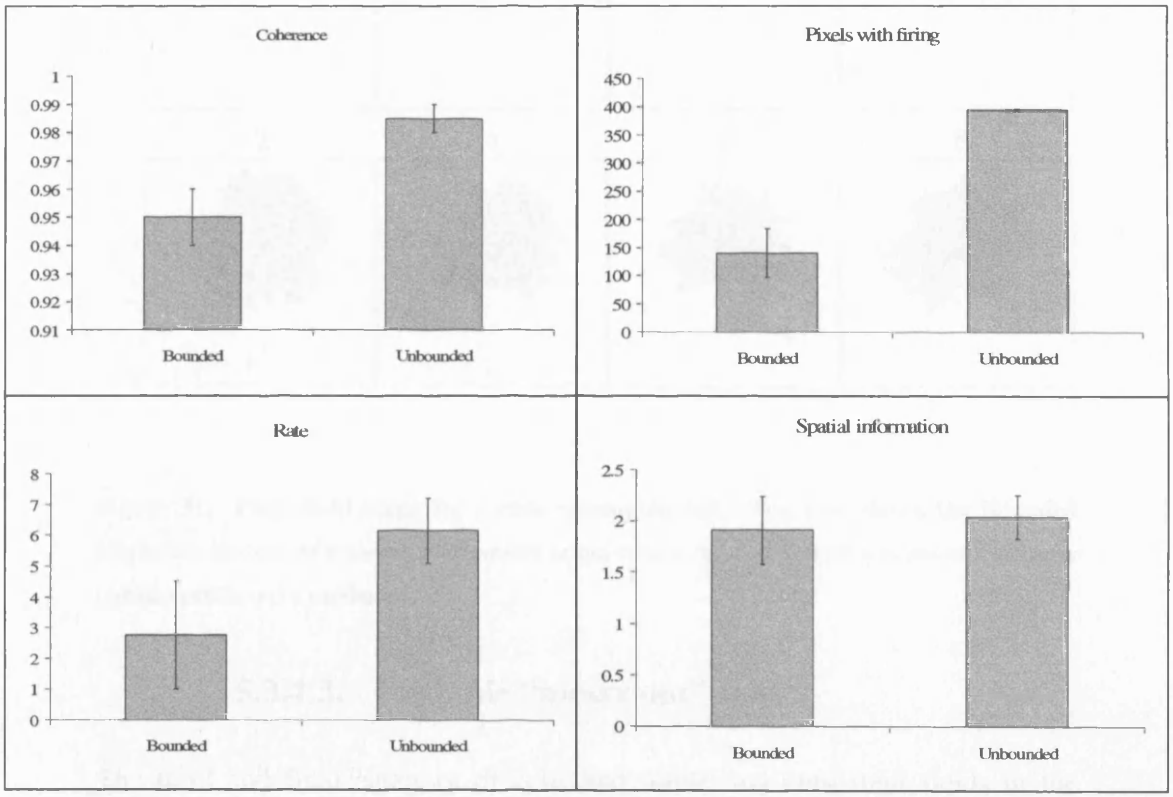


Figure 30. Rate, firing area, coherence and spatial information measures for a rate-remapping cell.

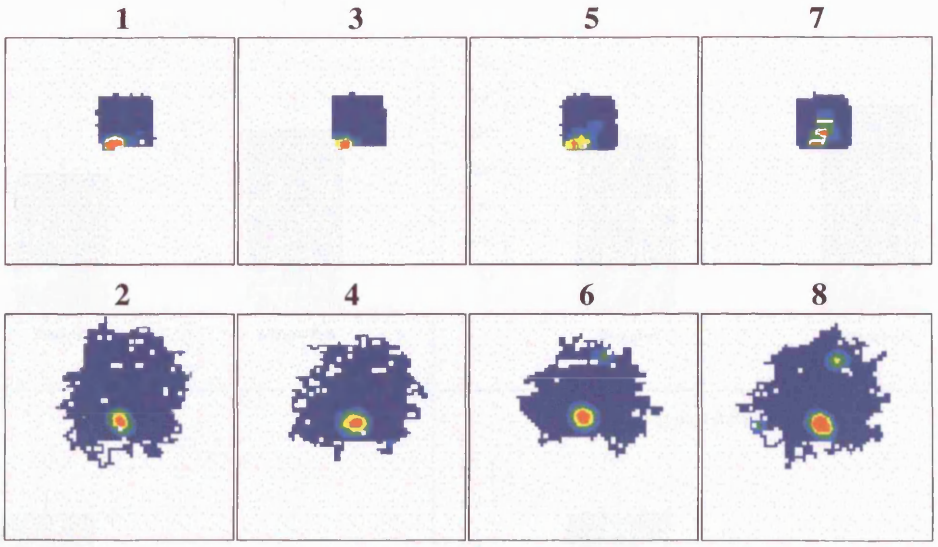


Figure 31. Place field maps for a rate-remapping cell. Top row shows the bounded trials, the bottom row shows unbounded trials where the South wall was moved. Empty (white) pixels were unvisited.

5.3.4.3. Unstable "messy out" cells

The third and final category of cells had stable and consistent fields in the Bounded box conditions and unstable or messy fields in the Unbounded conditions (n = 3). An example of this response is shown below in Figure 33. A paired t-test revealed a significant difference between the bounded and unbounded conditions in terms of coherence ($t(10)=4.67$, $p<0.001$). There was also a significant difference for the number of pixels that contained firing between the bounded and unbounded conditions ($t(10)=16.6$,$p<0.001$). There were no other significant differences on any of the other measures (see Figure 28, and Figure 32 and Figure 33 for examples).

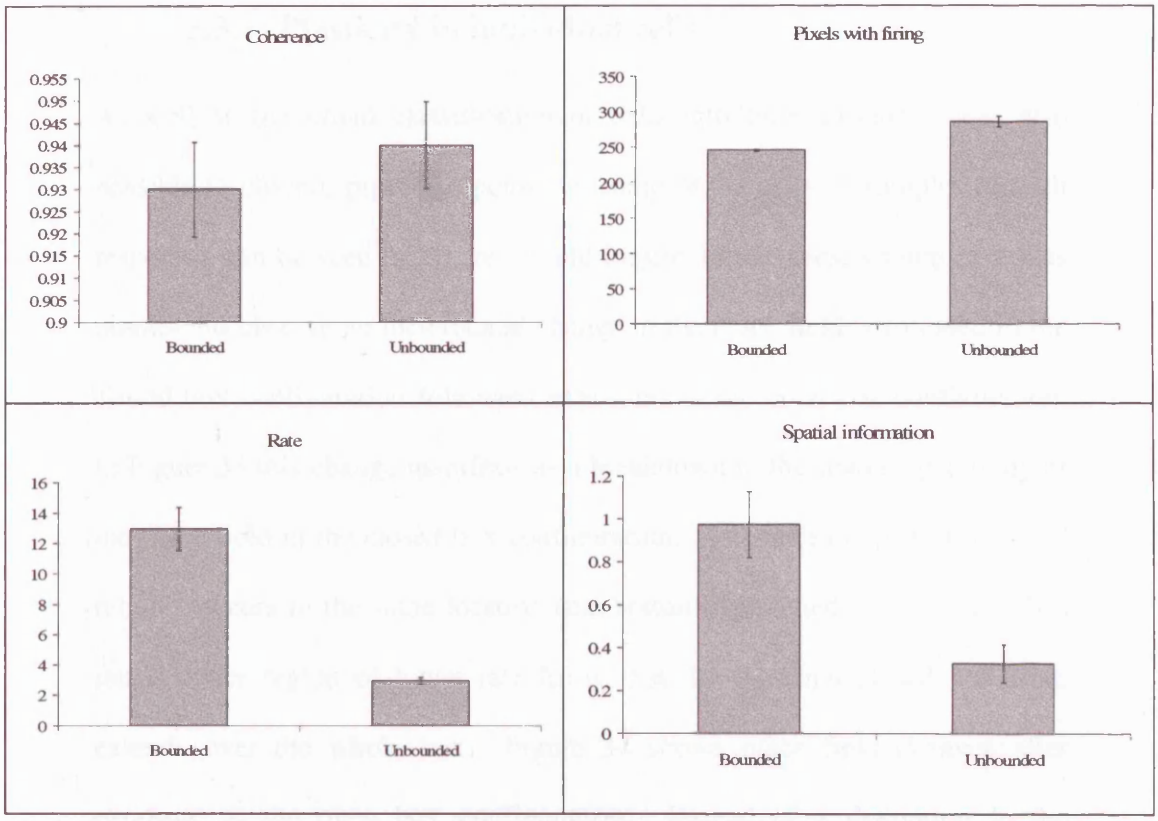


Figure 32. Rate, firing area, coherence and spatial information measures for an unstable cell.

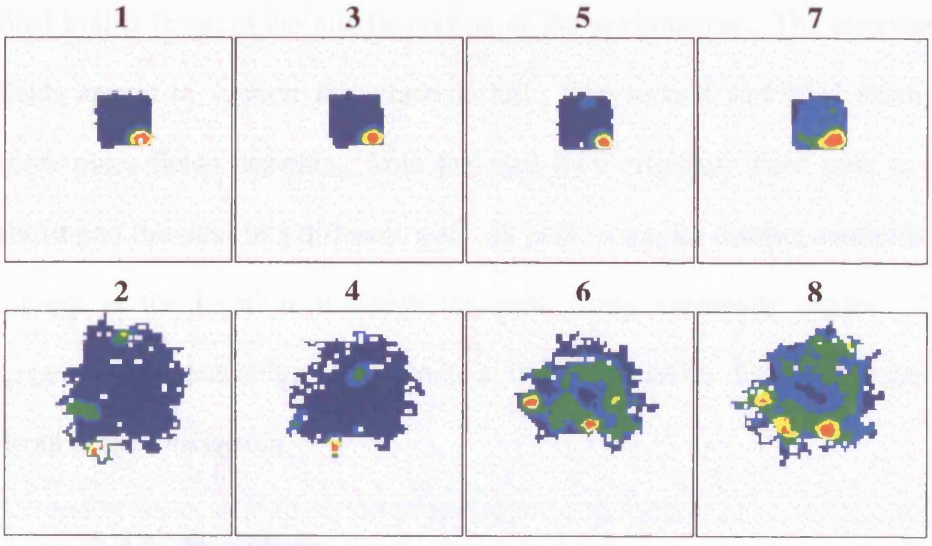


Figure 33. Place fields for the unstable cell shown in Figure 32. Top row shows Bounded conditions, bottom row Unbounded conditions. The South wall was moved out. Empty (white) pixels were unvisited.

5.3.5. Plasticity in individual cells

As well as the broad classification of cells into three groups it was also possible to observe plastic responses in some of the cells. Examples of such responses can be seen in Figure 33 and Figure 34. In these examples it was possible to observe an incremental change in the place fields expressed in the closed box configuration following exposures to the open box configuration. In Figure 33 this change manifests as a breakdown in the spatial specificity of the place field in the closed box configuration. Although the peak firing still reliably occurs in the same location (the bottom right-hand corner) there is a much larger region of lower rate firing that, by the final closed box trial, extends over the whole box. Figure 34 shows place field changes after exposure to the open box configuration. Instead of a disruption to the specificity of firing a gradual shift of the firing field is seen. In the first example in Figure 34 the field detaches from the left-hand wall and by the final trial is firing in the middle portion of the environment. The intervening fields appear to support this gradual shift. The second and third examples show place fields detaching from the wall they originally fired next to and shifting to fire next to a different wall. In both examples distinct subfields are present in the location at which the peak firing eventually settles. This suggests a reorganisation of the inputs to these cells and is discussed in greater depth in the Discussion.

5.3.6. Histology

Electrodes in all rats were confirmed to have been placed in region CA1 of the hippocampus. This was additionally confirmed by electrophysiological

criteria (see General Methods).

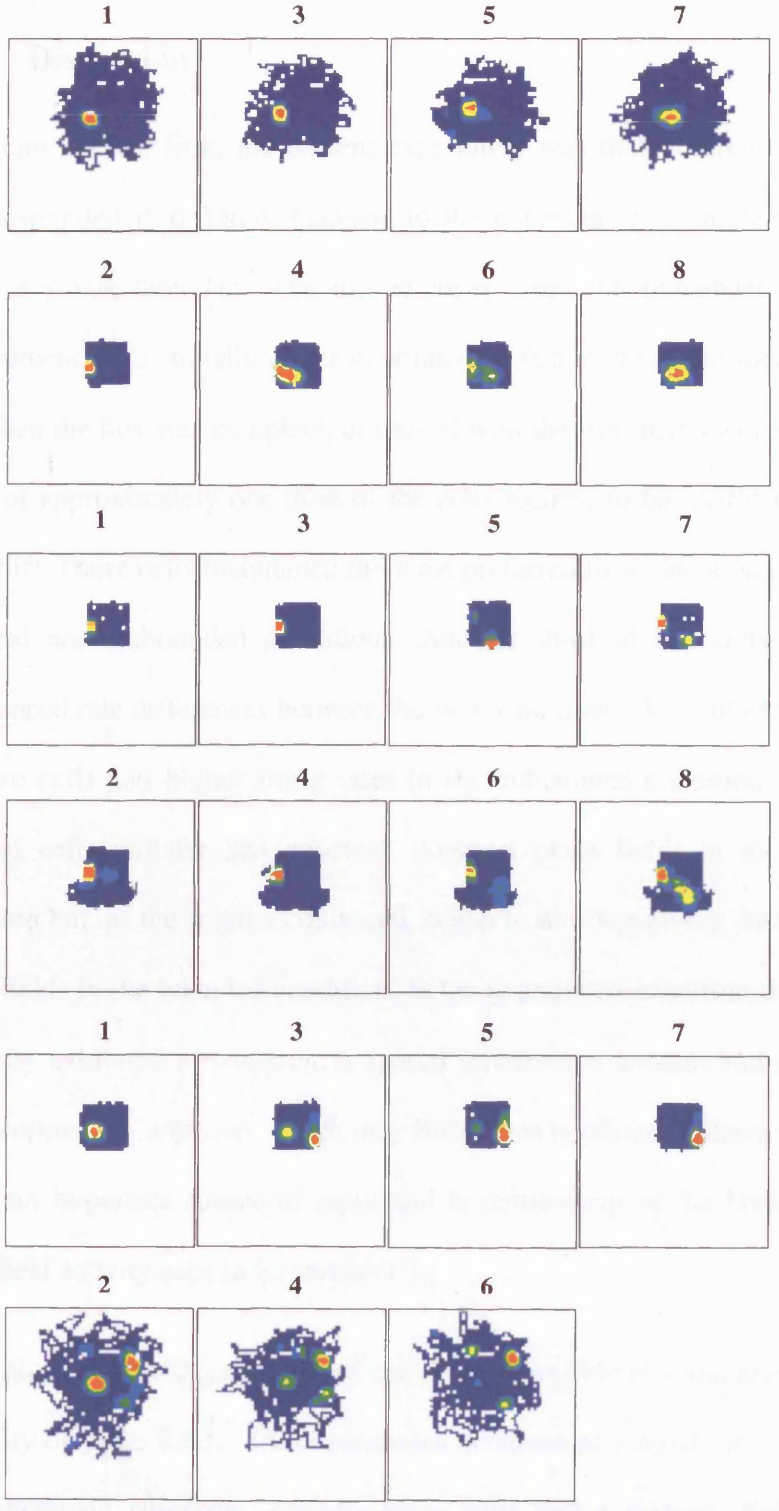


Figure 34. Examples of plasticity in the closed box configuration following exposure to the open box configuration.

5.4. Discussion

The main finding from the current experiment was that different groups of cells responded in different manners to the extension of a single boundary. When a single boundary was moved away from the remainder of a box environment cells initially either continued to fire in the same location they had when the box was complete, or moved with the wall that was moved. The fields of approximately one third of the cells seemed to be unaffected by the wall shift. These cells maintained the same preferred firing location in both the bounded and unbounded conditions. Another third of the cells exhibited pronounced rate differences between the two conditions. Without exception all of these cells had higher firing rates in the unbounded condition. The final third of cells initially had coherent, compact place fields in the bounded condition but, as the session continued, began to develop messy, less coherent firing fields in the bounded condition. In the unbounded condition these fields generally exhibited a reduction in spatial information content and coherence and increased the area over which they fired. This implies that these cells were losing an important source of input and is reminiscent of the breakdown in place field activity seen in Experiment 1.

In addition to these three groups of cells it was possible to sometimes observe plasticity of place fields. These responses occurred as a result of exposure to the unbounded condition. Initially these cells had a primary field in one location in the bounded box that, following exposure to the unbounded box environment, gradually developed a secondary field in a different location. With subsequent iterations of the bounded/unbounded conditions, the primary field became the subfield with less strong firing and the secondary field

assumed a higher firing rate. Of the three cells in which this response was seen, two had similar processes occurring in the unbounded condition and the final cell had a place field that broke down and developed multiple subfields in the unbounded condition, whilst exhibiting such a plastic response in the bounded condition.

Overall the aim of this experiment was to see if would be possible to decompose the boundary vector inputs that a given cell receives. The different responses seen to wall movements provide insight into the nature of these inputs. Therefore, the group of cells that failed to respond to any of the wall shifts can be said to be unresponsive to the particular wall that was moved and must instead be driven by some combination of the remaining walls. Although no formal analysis was carried out it appears that these cells generally had fields that were further from the walls than the other two classes of cells. A manipulation that could have been carried out would be to move out other walls from the box (so that only one wall at a time is disconnected from the main box) in an attempt to decompose the inputs that these cells received. However, in order to ease the interpretation and mitigate the effects of additional plasticity only one wall was moved per session[9]. One of the consequences of the boundary vector cell model is that boundaries nearer to the peak firing of a cell will tend to have more influence than those further away. Most of the cells in the unaffected group (n=3) had fields that were distant from the wall being manipulated (although no explicit measure of this was made). However one of the cells had a field that was abutting the wall that

[9] In every session except one the South wall was moved away from the local box environment. In that session, the East wall was moved away. No differences were noted between the South and East wall movements.

was moved. This cell did not display consistent changes in rate, nor broke down in terms of coherence or stability following the movement of the boundary. Instead the field remained coherent and moved with the wall that was being moved (see Figure 35 and also (Rivard et al., 2004). The mean correlation for bounded/bounded comparisons was 0.86, for the unbounded/unbounded comparisons 0.87 and for the bounded/unbounded comparisons 0.48. This suggests the maps in the two conditions were different (in this case identical although shifted) and that this cell was receiving strong boundary inputs from the wall that was being manipulated (the South wall).

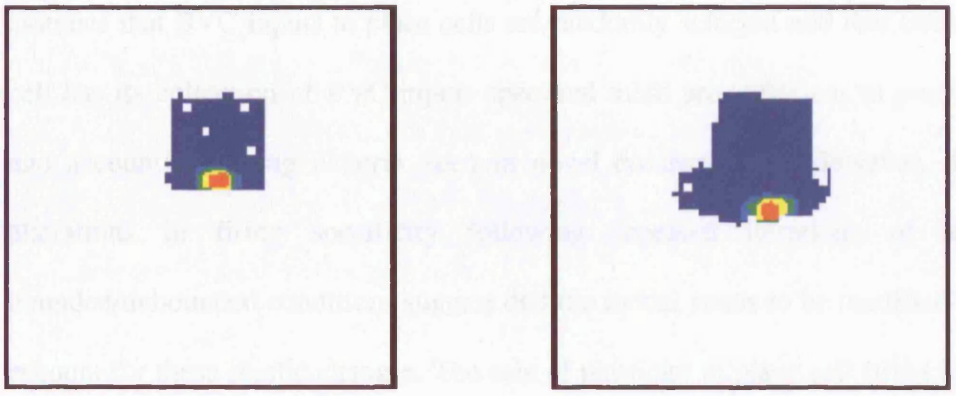


Figure 35. Example fields from the cell that followed the wall movement to the South. The bounded trial is on the left, the unbounded on the right. White pixels indicate areas not visited by the animal.

Approximately a third of the cells exhibited a rate remapping between the two environments. In all cases the rate was higher in the unbounded condition than the bounded condition. There was no evidence for a development of the firing rate i.e. no steady increase in firing rate was seen across subsequent trials. Thus it seems likely that plasticity was not involved in such a rate remapping, rather that either new inputs were recruited or the existing ones were driven

harder. When viewed in light of the BVC model it seems probable that the set of BVC inputs to the cell remains the same. If this were not the case then the model (and the experimental data that support it) would not be able to account for the firing patterns of cells when an animal is introduced into a new environment. If the BVC inputs are thresholded Gaussian tuning curves attached to the walls of the environment then, when the wall is moved apart from the rest of the box, this causes the sum of the BVCs to be more than it was when the box was bounded. The net effect is to see an immediate increase in the firing rate when the box is unbounded.

The results described so far are consistent with the BVC model. The model assumes that BVC inputs to place cells are randomly selected and that once a cell has its collection of BVC inputs specified these are sufficient to predict and account for firing patterns seen in novel environments. However, the alterations in firing specificity following repeated iterations of the bounded/unbounded conditions suggest that the model needs to be modified to account for these plastic changes. The role of plasticity in place cell firing has been documented in several experiments (Agnihotri et al., 2004;Kentros et al., 1998). Furthermore, experimental data from longer-term place cell recordings show that place cells can undergo a slow form of remapping resulting from accumulated plastic changes over several weeks (Lever et al., 2002b). The form of remapping seen in the Lever et al., result is reminiscent of the plastic changes observed in the current experiment with the fields that shift their focus of firing from a primary field to a secondary one over the course of several rounds of bounded/unbounded trials.

Results such as Lever et al., (2002), Kentros et al., (1998) and the present experiment suggest that in order to be a more complete description of place cell firing the BVC model needs to take plasticity into account. Such a reformulation of the model has recently been proposed based in part on the data generated from the current experiment (Barry et al., 2006). The reformulation of the model uses a modified Hebbian learning rule to update the weights that link the BVC inputs to place cells. The particular rule implemented is the Bienenstock-Cooper-Munro rule (BCM) (Bienenstock et al., 1982). As discussed in the General Introduction, the BCM rule allows both LTP and LTD to occur depending on the state of the postsynaptic neuron, with a sliding threshold that determines which of these two processes takes place. The BCM rule has previously been tested, alongside two other Hebbian-based rules, and was shown to be the rule most able to account for the gradual separation of two place cell representations (Fuhs & Touretzky 2000, and see Experiment 3 Discussion for more details).

As mentioned above, the recent reformulation of the BVC model uses the BCM rule to update the weights from the BVCs to the place cells (Barry et al., 2006). The learning occurs iteratively and weights are updated according to pre- and postsynaptic firing, place cell firing is re-calculated, weights are updated and so on. Two different environments were simulated, a square and a rectangular environment, with the rectangular environment representing the unbounded condition. Firing outside the area described by the environmental boundaries was not simulated. The results were generally consistent with the experimental data reported here. Fields present in both environments remained stable, maintaining the same positions relative to the environmental

boundaries. A small number of cells (~10%) were initially inactive on first exposure to the bounded environment, were subsequently active in the unbounded environment and thereafter had a field in the following bounded trials. This is a direct consequence of the BCM rule. When they are active in the unbounded environment the BVC connections to the place cells become strengthened such that when further bounded trials are run the cell is now able to exceed threshold. Such an effect was seen in the experimental data (n=4). The model only produced one cell that ceased firing (out of 100 simulated cells). This was an effect that was not seen experimentally.

The differences between the model and the experimental data add illustrate the shortcomings of the model. For example, cells that became active in the model did so following only a single trial in the unbounded environment. In practice it took several trials for inactive cells to become active. This may illustrate that the iterations that constitute a single trial in the model do not correspond to a single trial in the real world (a reasonably cosmetic difference). However, those cells that did become active did so in corresponding locations in the two environments, an effect that was always seen in the experimental data set.

The first two experiments attempted to assess the influence of geometry on place cell activity. The major findings from these experiments were that in order to form tight coherent fields, place cells need to be able to integrate input from many sources. Additionally, it was found in Experiment 2 that learning could occur amongst the putative BVC-to-place cell connections. Such learning processes form a major impetus to the final experiment. As discussed in both Experiment 1 and 2, it was possible that the geometric

changes made were being treated as a contextual change by the place cell system. This rationale is more fully justified in Experiment 3. With Experiment 3 no geometric changes were made to the environment. Furthermore the experiment was conducted such that the animal could view the spatial cues that constituted the experimental room. This would allow the place cells to integrate input from as much of the environment as possible and therefore exhibit more coherent and stable place fields. In the final experiment the phenomenon of remapping is assessed following incidental learning about the different positions of two environments in the experimental room. Following such learning an explicitly contextual change was made to the environment and the impact on place cells assessed. It was hoped that these manipulations would shed light on the nature of the contextual input arriving at place cells.

6. Experiment 3

6.1. Introduction

The previous two experiments have investigated the results of changes to the geometric configuration of an environment. Returning to the definition of geometry provided by (Gallistel, 1990);

> A geometric property of a surface, line, or point is a property it possesses by virtue of its position relative to other surface, lines, and points within the same space. A nongeometric property is any property that cannot be described by relative position alone. (p.212)

The current study is driven by two lines of experimental finding that explicitly manipulate such non-geometric properties. The first comes from work by Bostock, Muller & Kubie (1991) where a non-spatial change was made to the recording environment. The recording apparatus was a grey circular environment with a salient white cue card attached to the wall. Once stable cells were isolated and well quantified the colour of the white cue card was changed to black and another recording session was conducted[10]. Place fields in both conditions were well controlled by the cue card; rotations of the cue card led to rotation of the place fields. Following exposure to the black cue card remapped cells could be divided into two classes; rotational or complex cells. As mentioned earlier rotational remapping allows place fields to be superimposed by a simple rotation of the place field. Complex remapping is

[10] Note that a change in "colour" refers to a change in monochrome grey levels and not hue. Throughout the rest of the current experiment colour is used in this sense and it is *not* implied that the rats are using real colour cues.

where cells switch on/ off, or shift their fields to new locations. The time course of this remapping was such that cells recorded during early exposures to the black cue card showed predominantly a rotational remapping whereas those recorded during later exposures (after the first two exposures) tended to show complex remapping. Of the 9 pairs of cells recorded simultaneously all showed either rotational or complex responses to the cue card change i.e. rotational and complex responding cells were never recorded together. Repeated exposure to the two environments led to a discrimination being formed between the two environments such that the firing patterns in each environment became distinct. Interestingly once cells within a rat started to discriminate the two environments, all future cells recorded from that animal had different firing patterns in the presence of the black and white cards. Moreover, when the cells changed their firing patterns, the change was an instantaneous, all-or-none event.

Several insights can be taken from this experiment. First it is the demonstration that although the primary correlate of place cell activity is location, place cells can also be modulated by non-spatial environmental aspects such as colour. The other important finding is the experience-dependent change in place cell activity, suggesting that the inputs to place cells may well be plastic. It seems that with increasing exposure to the black card cells moved from a rotational remapping to a complex remapping. Furthermore the development of a new and different map (as opposed to just a rotationally equivalent one) in the presence of the black cue card did not interfere with the already established map in the white cue card environment. That the place cell representation can be modified with experience fits nicely

with evidence showing hippocampal involvement in learning and memory (see General Introduction). Indeed, the authors speculate that small changes in the sequence of exposures to the black cue card could "provide evidence that the changes in place cell firing bear a marked resemblance to learning." (p.204).

The other main impetus for the current study comes from work by Skaggs & McNaughton (1998) and Jeffery (2000). In the Skaggs & McNaughton study the apparatus consisted of two adjacent interconnected boxes that were made to be as identical to each other as possible. Rats were trained to forage for food reward and to thoroughly explore first one box and then the other. In all recording sessions except for a probe trial rats were placed in the North box first. After they were judged to have sufficiently explored the North box food was scattered into the South box and the rat moved (independently) to the South box. During a probe session on the final day of recording, instead of being placed directly into the North box first the rats were placed into the South box first. The intention of the study was to see whether two highly similar environments would be represented as separate, distinct place cell maps or as identical ones.

The results from this experiment were mixed. Data was collected from four rats, each of which had cells that displayed different patterns of responding. The main findings were that the maps activated in the North and South boxes were neither identical nor completely distinct. For all of the rats at least three days of recording was performed, each day consisting of two sessions of 12-15 minutes. One pattern of responding that was never seen for any place field was that if a field was confined to one box location in the first session then this

field never shifted to fire the opposite box location in the second session. Despite this it does seem that in some instances there were cells whose fields switched on/off across sessions in either location (5/50 cells from two sets of 25 simultaneously recorded cells from two different rats). There were also significant differences between rats in terms of the degree of similarity between North and South maps. The first rat had more dissimilar representations for the North and South locations whereas the other three had more similar representations of the North and South locations. Interestingly, a significant variability in the distributions of the North-South (N-S) correlations across days for three of the rats was also found. For the first rat the N-S correlations were indistinguishable across days. The N-S distributions for the other three animals varied significantly across days however. Despite this there was no evidence for either an increase or decrease in the level of N-S correlation across days. The authors concluded there was no evidence to support the idea that there were changes in the place cell response as a function of experience. This is in clear contrast to the results from Bostock et al. A possible explanation for the lack of experience-dependent changes seen here is the highly similar appearance of the two boxes and the limited amount of experience the rats had of them. That the boxes were highly similar is attested to by the high proportion of cells showing indistinguishable responses in both locations. Also, although not explicitly stated in the paper, it appears that the longest recordings were performed for was 10 days at two sessions per day (rat 1: this excludes a 3-4 day pre-training period not included in the main analysis). This can be contrasted with the Bostock et al., study where cells were recorded from for up to 106 days and 40 separate sessions. So it is more

likely that experience-dependant changes would be seen with Bostock et al., than with the Skaggs & McNaughton study.

Data from the probe session were also ambiguous. Here the rat was introduced to the South box first where previously it had always been introduced to the North box first. In one of the rats the North map was instantiated upon initial exposure to the South box. It was only when the rat had moved from the South to the North box (where the North map was correctly activated) and back to the South box that the correct South map was recalled. The authors interpreted the results in terms of the animals "expectations" of its start location in the apparatus and of a mechanism (possibly path integration) that allows the animal to remember its movements through the corridor.

Other data relevant to the current study comes from a pilot study by Jeffery (2000). This is a similar study to Skaggs & McNaughton (1998) with the difference that the box locations were slightly overlapping (one of the boxes was translated ¾ of the length of the other box) and were not connected by an adjoining corridor. Following repeated experience of the two overlapping locations it was found that place cells *gradually* began to discriminate both boxes by forming different place fields in the two box locations (Jeffery, 2000). This addresses one of the shortcomings of the Skaggs & McNaughton study mentioned above. It is possible that with greater experience of the two box locations that the cells in the Skaggs & McNaughton study may well have started to form heterogeneous place fields in the two locations. As the two boxes are so similar in appearance it is feasible that it would take an extended

period of time for pattern separation to occur. Data from the Jeffery (2000) study addresses this concern by looking at the long-term patterns of place cell activity. Over time it was found that the ratio of discriminating to non-discriminating cells increased. That is, place fields expressed in each of the boxes were different – a remapping had occurred. As the only difference between the two box locations was the distal cues (the influence of path integration and local cues were controlled for by disorienting the rat between each trial and washing the wall and floors with ethanol) it seems that the cells were being progressively more influenced by these distal sources of information. Additionally there must have been a concomitant weakening of the influence exerted by proximal cues. If the inputs from these cues were not becoming weakened then the proximal cues would have always been enough to drive the cells above threshold and the cells would be non-discriminating (as the proximal cues are effectively identical). The equilibrium between the relative levels of excitation impinging on cells also makes sense in terms of the observed firing rate. If the drive provided by the proximal cues was maintained at a constant level and the drive from the distal cues increased over time then this would manifest as an overall, gradual increase in the firing rate of the cell. Such a change was not seen. What happened more commonly was that a given cell would stop firing in one or other of the boxes whilst maintaining a field in the other box. This is similar to the complex remapping seen in the Bostock et al., study. So it appears that the input provided by the proximal or distal cues alone was no longer enough to cause the cell to exceed threshold. Instead a combination of proximal and distal cues was necessary to cause the cell to fire.

Such observations of a change in neural activity as a result of a rat's experience of an environment have also been echoed more recently in an experiment by Lever et al., (2002). Part of the rationale for their experiment was that in order to capture experience-dependent changes in place cell activity it is necessary to record from the rats' initial exposure to an environment through to the termination of the experiment. To this end, place cells were recorded in square or circular boxes over a period of many days. The place fields in the circle and square box were initially similar but incrementally diverged. Individual differences were again seen between animals – here it was the time taken for the divergence in firing pattern to occur that varied. Furthermore the pattern of remapping seen in this experiment was partial (with some cells showing the same fields in the two locations and others showing different fields), as opposed to the complete remapping seen with Bostock et al. The patterns of activation also showed generalization across boxes of the same shape but made of different material. Additionally the change in place field activity was persistent, lasting at least a month, and experience dependent (i.e. time alone did not account for the shape-specific firing patterns). The authors proposed that the long-term, experience dependent divergence of place cell representations seen in this experiment is commensurate with a system that underlies long-term incidental environmental learning.

Based on the results from the Jeffery (2000) and Lever et al., (2002) studies it appears that place cells can acquire discriminative information that allows them to distinguish between two similar environments. Because of the known involvement of the hippocampus in the processing of context an interesting

question to ask is how a acquired discrimination transfers between different contexts. Would information acquired in one context be specific to just that context? Alternatively, would the acquired information be shared across different contexts? In order to address these questions, in the current experiment place cells were recorded in alternating trials in two neighbouring boxes. As described above this is a procedure that gradually induces remapping in some cells (Jeffery, 2000;Skaggs and McNaughton, 1998). When a discrimination between the two boxes was evident as a remapping, the context of the two boxes was altered by changing the box colour from black to white or vice versa and then alternating trials were continued as before. One of three possible consequences was predicted to occur. First, place cells might continue to discriminate the two locations in a similar manner to the cells in the Lever et al., study where cells generalized across boxes of the same shape but made of different types of material. This would suggest that the learning that had occurred was a property of the spatial mapping system as a whole. Second, it is possible that only those cells that were *active* and discriminated locations in the familiar context would continue to discriminate locations in the novel context. This would imply that the discrimination that occurred is a function of prior activity in the familiar context. Third, place cells active in the novel context might fail to discriminate locations at all.

6.2. Methods

6.2.1. Subjects

The data from 4 adult male Lister Hooded rats (350-420 g) was used in this experiment. Handling, feeding and photo-periods were as described in the General Methods section.

6.2.2. Surgery

As described in the General Methods.

6.2.3. Recording room and local environments

Initially, the floors and walls of the two box environments, North and South, were always the same colour (herein the environment animals were first exposed to will be called the familiar context; black for three rats, white for one rat). Rats were exposed to the familiar context until remapping occurred; this took between 3 to 10 days. The walls of the boxes were made from wood and were 70cm long, 35cm high and 1cm thick; a given box therefore formed a 70x70cm square. One side of a wall was painted black and the other white – when the context was changed the walls were switched to show the other colour. Alternating 4-minute trials were run in the two locations; trials always started in the North position. Distal cues were available to the rats in the form of posters along the top edge of the experimental room wall and floor-to-ceiling curtains along the East wall (see Figure 36). A minimum of at least 3 trials in each location were run each day; usually 8 trials in each location were run (a total of 16 trials overall). Between each trial the walls and floor of the environments were interchanged so the rats experienced a unique configuration of local cues (marks on the walls, olfactory cues on the floor

etc.) during each trial. The purpose of this manipulation was to ensure that the local cues would not be a valid reference for discriminating location (Save et al., 2000). This inter-trial interval was approximately 2-3 minutes long. Once remapping between the two locations had occurred (see below for how this was quantified) in the familiar context, the colour of the walls and floors in *both* box locations was changed to the other colour, to create a second context (herein the second context will be called the novel context). Throughout the whole experiment the boxes remained in the same absolute location within the experimental room. Alternating 4-minute north/ south trials were run, as before. The walls and floors were again interchanged between each trial.

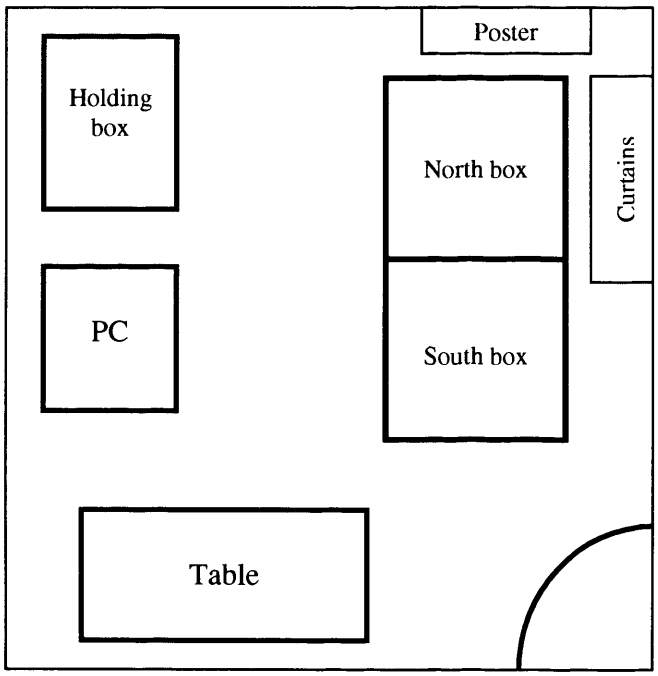


Figure 36. The experimental recording room used for Experiment 3.

6.2.4. Quantifying remapping

Remapping was assessed using a correlation procedure as follows. Following a change to the environment (location, colour or both), cells were considered

to show remapping if the location of the field changed or the cell started or stopped firing (cessation of firing was classified as an averaged firing rate below 1.0 Hz). In terms of the present analysis, a cell that either stopped or started firing upon a change of condition was coded as having rate remapped and no further correlation analysis was conducted (as there were no data to correlate with). For each cell with a field in more than one condition, remapping was assessed as follows. A smoothed firing rate map was generated for each condition where the field was present. Each map was then decomposed into a 64x64 element matrix. Then each pixel in one map was correlated, by a Pearson's correlation, with its equivalent pixel (in terms of its absolute position) in the other map. Pixels that had a zero firing rate in both maps were discarded so that all the common areas where the cell was not active did not artificially inflate the correlations (Jeffery and Anderson, 2003). In one of the rats, the majority of place fields in two conditions were only distinguished by a difference in their respective firing rate (i.e. the cells were firing in the same location in two different location conditions). A difference in firing rate was assessed by a one-tailed t-test comparing all of the same-condition rates with all of the different-condition rates (see belowGeneral Methods for justification of the use of a parametric test). Again, a significant difference was taken to indicate that remapping had occurred.

In order to assess how cells responded to changes in the colour, location or both properties, several different criteria were evaluated. Initially cells were assessed for remapping in terms of turning off – designated as "rate remapping". If the cell fired in both conditions, the firing location was tested to see whether it had changed ("location remapping"). This was assessed by

correlating the first firing rate map with the second (see Experiment 3 Methods). A high correlation indicates a highly similar firing location and hence no remapping, while a low correlation indicates a change in the firing location and hence remapping. Two kinds of correlation were performed. Within-condition correlations tested how much a place field varied between recording sessions composed of the same stimuli (location and box colour), and served as a measure of the stability of the place cell representation. Between-condition correlations were made for recording sessions differing in the location of the box, the colour of the box or both, and provided a measure of remapping.

For a cell that was active over sixteen 4-minute trials (eight alternating North/ South trials in the familiar context and eight in the novel context) in a session, the following correlation coefficients were produced (and see Figure 37 for schematic of the different correlations):

- Within condition correlations:
    - North [familiar context v. familiar context] – 6 correlation values
    - South [familiar context v. familiar context] – 6 correlation values
    - North [novel context v. novel context] – 6 correlation values
    - South [novel context v. novel context] – 6 correlation values

- Between condition correlations:
    - North [familiar context v novel context] – 16 correlation values
    - South [familiar context v novel context] – 16 correlation values
    - Familiar context [North v. South] – 16 correlation values
    - Novel context [North v. South] – 16 correlation values

The within condition correlations for each context condition (North [familiar context v. familiar context] and South [familiar context v. familiar context]) were averaged to produce a single correlation coefficient for each context, designated familiar context within-condition correlation coefficient or novel context within-condition correlation coefficient. Typical within-condition correlations for context were around 0.6.

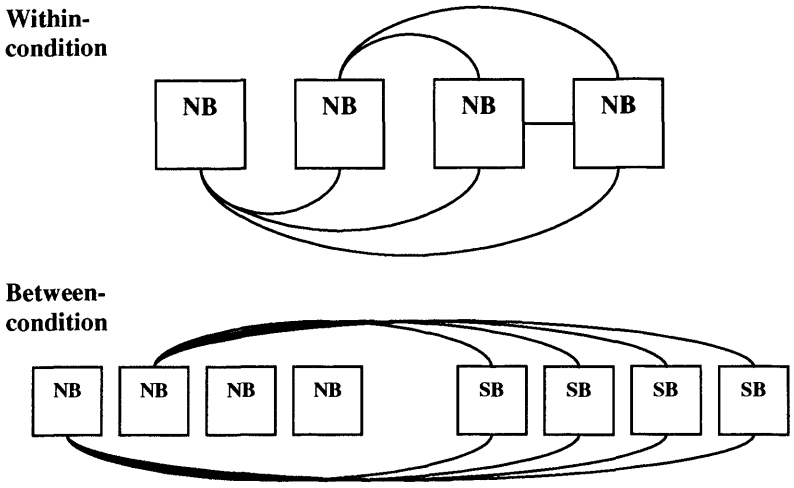


Figure 37. Schematic of the analyses performed for the within and between condition correlations. Top shows within-condition correlations, bottom shows between-condition correlations. Note that the within-condition correlations produce 6 correlation values, the between-condition correlations produce 16 values in total. Only the first 8 between-condition correlations are shown for clarity. NB – North Black; SB – South Black.

The between-condition correlations for each context showed the relationship between maps in the two different locations in one context (North-black v. South-black). For a cell that had remapped the overall between-condition correlations for context were roughly +0.1- +0.2. The between-condition correlations for each location showed the relationship between maps in the same absolute location in the experimental room, but under different context conditions (i.e. North-black v. North-white).

In order to normalise the distributions of the correlation coefficients (so parametric statistics could be used), the data were transformed using the Fisher r-to-Z transform according to the following formula:

$$Z = 0.5\log_e(1+r/1-r)$$

All data analyses were performed on these transformed scores.

6.2.5. Histology

As described in the General Methods.

6.3. Results

A total of 50 cells were recorded from across all conditions from the four animals used in this experiment (n = 13, n = 9, n = 17, n = 11) that satisfied the acceptance criteria detailed in the General Methods. The proportion of cells active in the different conditions was as follows: 39 active in the familiar context, 32 active in the novel context and 17 active in both contexts.

The within-condition correlations for the four different conditions (familiar North, familiar South, novel North and novel South) were approximately normally distributed around a mean of 0.84 (± 0.02) and did not differ between conditions [$F(3,116) = 1.90$, NS]. This value (0.84) is useful as a measure of stability of firing within a condition. These values were grouped together for further comparison using t-tests and analysis of variance.

6.3.1. Remapping to colour

As previous studies have also found (Anderson and Jeffery, 2003;Bostock et al., 1991;Kentros et al., 1998) changing the colour of part or all of the recording box from black to white caused a change in the firing patterns of the place cells (remapping), which was quantified using the correlation analysis described in the Methods. Firing rate maps were compared between familiar and novel contexts in the North and South locations. The mean between-colour correlations were 0.27 (± 0.04 SEM) and did not differ for North and South locations (two-tailed $t(34) = -0.40$, NS). The low coefficient values indicate that the place fields in one colour bore little relation to place fields in the other colour, as was expected, and this remapping was as strong for the North box location as for the South.

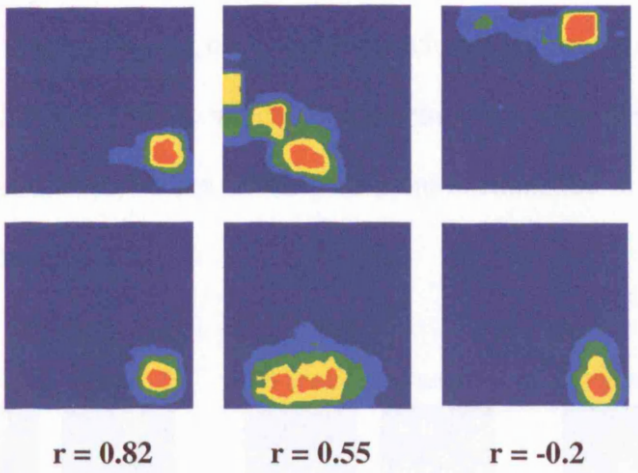


Figure 38. Example smoothed firing rate maps and their associated correlation scores. Each column shows a different cell in two different conditions. Underneath each column is the correlation value for the correlation between the two firing rate maps.

6.3.2. Remapping between North and South

Remapping between North and South was quantified using the between-condition correlation for each colour, which reveals the relationship between place fields recorded in the same colour box but in different absolute locations in the experimental room (e.g. black-North v. black-South). Remapping between North and South was strong in the familiar context, with a mean between-location correlation coefficient of only 0.11 (± 0.01 SEM), which is even lower than the correlation between boxes of different colours and indicates substantial remapping. When two or more cells were recorded at the same time, all the cells exhibited the same behaviour: i.e., all remapped or all failed to do so (as with Bostock et al., 1991).

Overall, this indicates that the cells were discriminating the two locations on the basis of the distal cues. Cells switched on and off (one rat), shifted the locations of their firing fields (two rats) or changed their rate substantially

(one rat). This rat-specific behaviour is interesting, and is discussed in greater detail below. Fields were not observed to stretch and deform as occurred with O'Keefe and Burgess (1996), which suggests the distal cues were acting in a different manner from the box walls. This point is examined in greater detail in the Discussion.

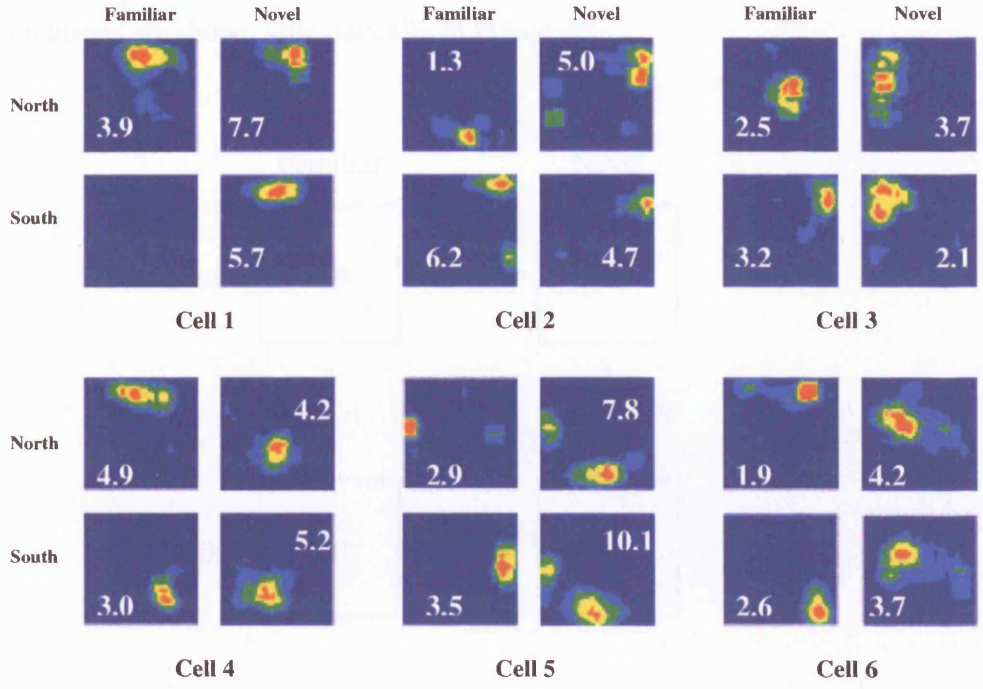


Figure 39. Place fields from six cells recorded in the North and South box locations, first in one context ('Familiar') and then in another ('Novel'). The peak rate (Hz) is shown alongside each place field in white. Although these cells remapped in the familiar context, either by shifting their fields or by switching off in one of the boxes, they failed to transfer this discrimination to the novel context.

Despite showing robust remapping in the familiar context, many cells failed to discriminate these same locations in the novel context (Figure 39). This was borne out by the correlation analysis, which showed a between-location correlation coefficient of 0.54 (± 0.06 SEM). A one-tailed t-test confirmed a

significant difference between the between-location correlation coefficients in the two different contexts (t(58) = -2.26, p= 0.01). This indicates that cells in the familiar context condition had unrelated place fields between the North and South locations (i.e. had remapped and were now discriminating), whereas cells in the novel context condition had closely related place fields between the North and South locations. The correlations between the various conditions are shown schematically in Figure 40.

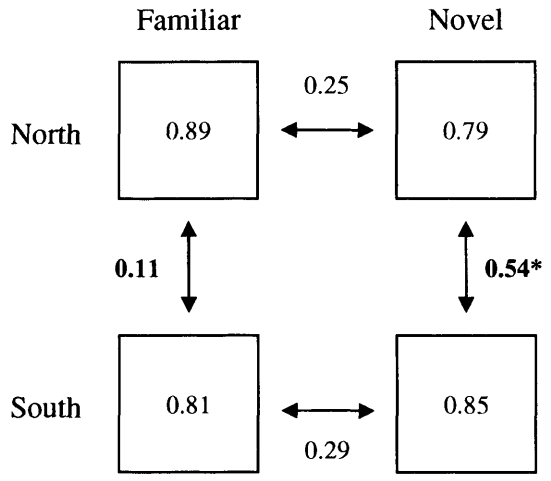


Figure 40. Schematic diagram showing mean within- and between-condition correlations for place cells recorded in the familiar or novel boxes in the North and South locations. Numbers within boxes indicate within-condition correlations; numbers next to arrows show between-condition correlations. The between-colour correlations (0.25 and 0.29) were not significantly different from the between-location correlation for location in the familiar context (0.11). However, comparing the between-location correlations for location in the familiar (0.11) and novel contexts (0.54) reveals a significant difference. Asterisk indicates significance at p<0.01.

The between-condition correlation value, while relatively high, was nevertheless significantly different from the within-condition value (t(98) = -3.91, $p < 0.001$), suggesting some degree of remapping. The reason for this result can be explained by the results from the third rat. Here cells remapped

extremely quickly in the novel context (see below for details).

6.3.3. Animal-specific remapping patterns

A closer examination of the pattern of remapping in individual animals revealed that there were clear differences between animals in the overall behaviour of cells. For two of the animals (rats 1 and 2), when cells remapped location they almost always did so by remapping their fields to novel areas of the environment (Figure 41). Of a total of 22 cells recorded from these two animals that remapped location, only 1 switched off, with the rest shifting their place field to a novel position. However, a different pattern was observed in another animal whereby approximately half (9/17) of the recorded cells remapped by either switching on or switching off (Figure 41). These differences in individual patterns of remapping were compared using a χ^2 test. There was a significant difference between rats in the type of remapping seen (either cells switching on/ off, shifting location or rate remapping) ($\chi^2 = 53.25$, $df = 6$, $p < 0.001$).

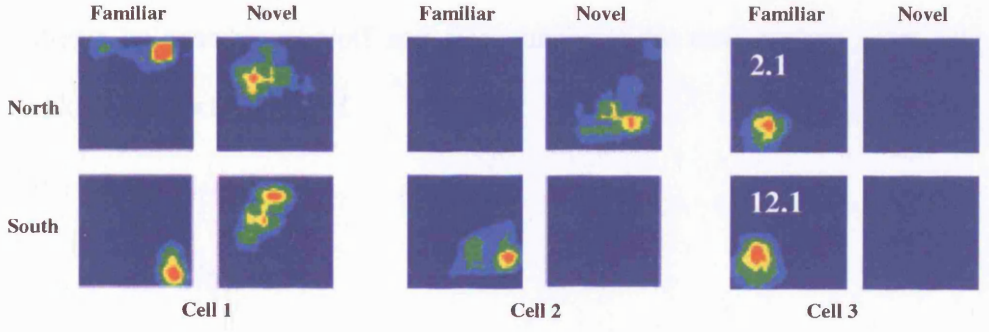


Figure 41. Animal-specific remapping patterns. Each place field demonstrates behaviour typical for the majority of cells recorded from that animal. Cell 1 remapped in the familiar context by shifting its field to a new place within the recording box. The fields in the novel context are highly similar. Cell 2 remapped by switching on/off, both

in the familiar and novel contexts. This cell was particularly interesting because it (unusually) expressed the same field in both contexts but in opposite locations. Cell 3 rate remapped between the familiar north and south locations and was inactive in the novel context. See text for a detailed discussion of the responses of cells 2 and 3.

Whereas the cells recorded from rats 1, 2 and 4 took time to develop a discrimination between locations in the familiar context and rarely, if at all, remapped location in the novel context, the cells recorded from rat 3 discriminated location rapidly in both contexts. As mentioned above this is the reason for the between condition correlation value of 0.54 (which lies between the average within condition value of 0.83 i.e. no remapping, and the 0.31 value for the between condition correlation in the familiar environment i.e. successful remapping).

Because of the rapidity with which cells remapped in rat 3 two additional manipulations were carried out with this animal. Here the rat was exposed to a third context consisting of a circular environment with corrugated cardboard covering the inner walls. The responses recorded from this one of the cells can be seen in Figure 42 below. The cell remaps both the familiar and novel contexts by switching on/off and also remapped the new context extremely quickly by switching on/off.

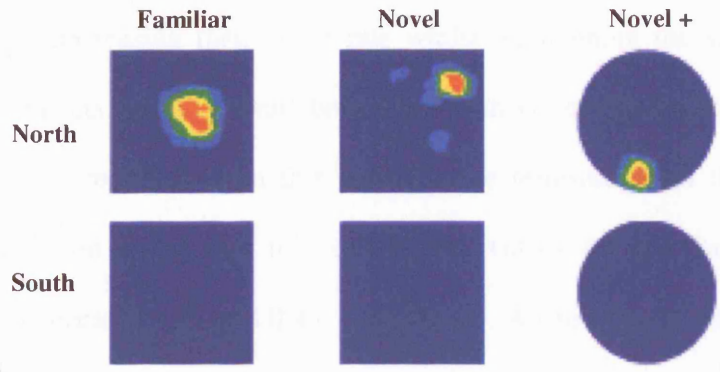


Figure 42. Place fields recorded following exposure to a third context in the rat that remapped the first two contexts extremely quickly. The newest context (Novel+) is also remapped instantly.

Another pattern of remapping was observed in a further animal (rat four). Here, instead of remapping fields to novel parts of the environment or becoming active or silent, the cells either increased or decreased their firing rate whilst their fields remained in the same location with reference to the box (Figure 41). In order to quantify the firing rate differences, t-tests were conducted on the firing rates of cells that had identical fields in the two box locations. Some cells expressed their high rate in the North box and some in the South, ruling out some general arousal or other factor specific to one of the box locations. Thus, this "rate remapping" seems qualitatively different from the more commonly observed switching on or off of fields (see Figure 43).

Because the cells recorded from this animal signified a change in location by increasing or decreasing their firing rate whilst maintaining the same place field, this impacts on the overall between-condition correlation for context. When the cells recorded from this animal were removed from the overall between-condition correlation for context, the values for the familiar and novel context became 0.09 and 0.43, respectively. A t-test conducted on these revised values revealed an even more significant difference between the between-condition correlation coefficients in the two different contexts ($t_{(47)} = -0.487$, $p < 0.0001$).

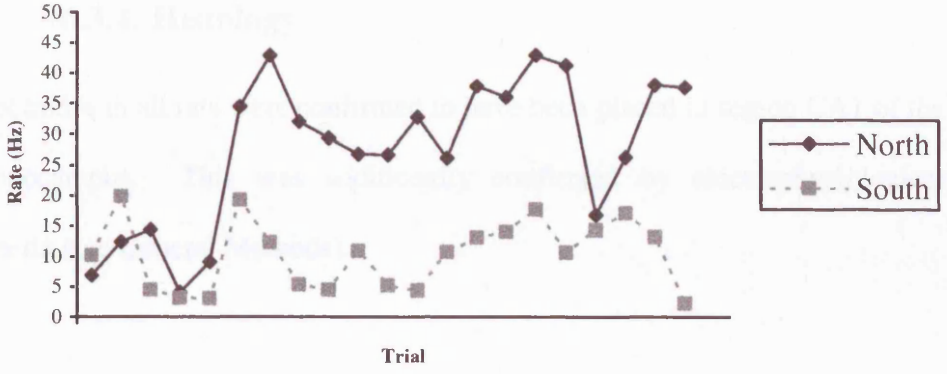


Figure 43. Differential rates in the familiar context for a cell recorded for four consecutive days from a rat with cells that primarily discriminated based on rate (7/ 11 cells). 8 sessions were run on each the first 3 days, 6 on the last day. The rate of the cell is significantly different in each box location (p<0.001).

One final observation is made which, while anecdotal, may shed light on the structure of the contextual signal reaching the place cells. This cell, shown in Figure 41 (cell 2), was recorded from the rat whose cells quickly learned to discriminate the locations in the novel context too. This cell expressed a

particular field in the South in the familiar context, and the same field in the North in the novel context. The possible significance of this observation is detailed in the Discussion.

These results thus show that newly acquired discriminative information about distal cues is expressed by place cells in a context-dependent manner. This indicates that the spatial inputs onto the cells can be modulated independently of each other and that the acquired inputs act at this level, rather than on the place cell itself. In the Discussion, a model is presented of how this might occur.

6.3.4. Histology

Electrodes in all rats were confirmed to have been placed in region CA1 of the hippocampus. This was additionally confirmed by electrophysiological criteria (see General Methods).

6.4. Discussion

The main finding from this experiment is that a discrimination acquired in one context is not passed to another, novel context. Rats were exposed to a situation where cells are known to gradually remap (Jeffery, 2000), which is taken as evidence that a discrimination has occurred. Rats were repeatedly exposed to two boxes differing only in their relative positions in the experimental room. The only consistent difference between these two locations was the view available to the rat of the distal cues outside the boxes. When cells remapped it was presumably due to this difference. This conclusion was also supported by the results of a pilot study (not reported here) that used a box with much higher walls (wall height = 70cm; twice the height of the walls used here) than those used in the present experiment. All other manipulations/ conditions carried out were identical to the current experiment. The cells recorded in the pilot never succeeded in discriminating the two box locations. Arguably, the view available of the distal cues from the box was too restricted. It is highly likely a discrimination never formed because the limited view of the distal cues from the two locations was not sufficiently different to support pattern separation of the environmental inputs.

The majority of remapping seen between the two familiar locations was what Bostock et al., described as complex - cells switched on/ off or shifted their preferred firing location. This occurred for three of the rats included in this experiment. Cells recorded from the fourth rat discriminated locations in the familiar context by expressing differences in firing rate. Here the place fields were topographically the same between different box locations but displayed large differences in terms of their firing rate. The significance of this finding

is discussed in more detail below.

Once the remapping between locations in the familiar context was evident, the context of the two boxes was altered by changing the colour of the boxes from black to white (or vice versa). This is known to produce a more instantaneous remapping (as opposed to the gradual remapping seen with repeated exposure to two different box locations) (Bostock et al., 1991;Quirk et al., 1990). Once again the type of remapping seen with this manipulation was complex – cells switched fields on/ off or shifted their field locations. Following the change in colour, cells always exhibited a complex and complete remapping and were never observed to maintain constant place fields whilst systematically displaying a difference in firing rate.

In general, neither cells that had successfully discriminated North from South in the familiar context, nor newly active cells, were able to discriminate between the two locations in the novel context. Thus the type of remapping seen here is different to that seen with Lever et al., (2002). With Lever et al., the remapping generalized across environments of the same shape but made of different material. Also, because cells failed to discriminate the novel context even when they were active in the familiar context, this suggests that remapping was not a product of prior activity. Extending this point, it does not appear that cells had over-learnt a response in the familiar context and were therefore unable to express a different response pattern. When exposed to the novel context previously discriminating cells underwent a complex and complete remapping, expressing wholly novel place fields. Additionally, on several occasions, exposure to the novel context resulted in new cells

becoming active that had not been active in the familiar context. These cells also failed to distinguish the novel context, further supporting the idea that remapping was not due to prior activity.

Therefore an interaction exists between remapping to location and remapping to the colour change. The nature of this interaction can be explained through a simple computational model where the inputs impinging on a given place cell are differentiated into geometric and contextual inputs. The geometric, or boundary inputs (Hartley et al., 2000) are responsible for selecting which particular field is expressed by a cell; the contextual inputs control which boundary inputs are expressed by the cells in a particular environment (see Figure 44). It can be seen then that a hierarchical relationship exists between these different input types with contextual inputs located at the top of the hierarchy. Boundary inputs are assumed to carry a purely spatial signal, free from any contextual signals (Anderson and Jeffery, 2003;Hartley et al., 2000). These are activated when the rat enters the environment and are driven by the walls present in that environment. Therefore the boundary inputs are modulated by the contextual inputs. This allows the individual fields of a cell to be switched on/ off independently of each other. Contextual remapping thus reflects the recruitment of a new set of boundary inputs by newly active contextual inputs.

6.4.1. The Model

The model presented below is an extension of the work presented in (Anderson and Jeffery, 2003;Jeffery et al., 2004). It starts with the observation that cells only began to discriminate between the two locations in the familiar

environment following repeated experience. The majority of cells discriminated location by either switching on/ off a field or forming a different place field. There are two possible explanations for this. The first is that the discriminative stimuli outside the box served an inhibitory role, thus causing place fields to switch off. The other is that they served an excitatory role (via LTP) with the overall level of excitation maintained through a synaptic scaling process, possibly involving LTD (see Turrigiano & Nelson, 2000). The latter explanation is considered more likely as LTP and LTD are well documented in the hippocampus and feedforward inhibition less so (cases where feedforward inhibition occurs in the hippocampus are documented are examined in greater length in the General Discussion). A diagram of how this process may occur is shown in Figure 44 and Figure 45. Although feedforward inhibition of pyramidal cell responses *has* been documented in the hippocampus, this occurred along the mossy fibre input from granule cells of the DG to CA3 pyramidal cells (Mori et al., 2004). It appears that a frequency dependent switch occurs such that at low levels of presynaptic input from DG to CA3 principal cells inhibition dominates. At higher levels of presynaptic input however a switch occurs such that excitatory input is facilitated, inhibitory circuits are depressed and the synaptic response (at the CA3 cell) becomes excitatory. Although low levels of basal firing are seen in the DG cells in this layer, the cells also display stable spatially selective fields that persist for days given the same environmental conditions (Jung and McNaughton, 1993). Given that the cells fire with increased frequency when the animal enters the place field then it is likely that the spatial input to CA3 is above the threshold required to make the postsynaptic response excitatory. Therefore, it is likely

that of the two scenarios described above (feedforward inhibition or excitation with scaling) that excitation with scaling is the likely mechanism for the discrimination occurring. Additionally, the frequency-dependent switch was described in CA3 and has not (yet) been documented in CA1.

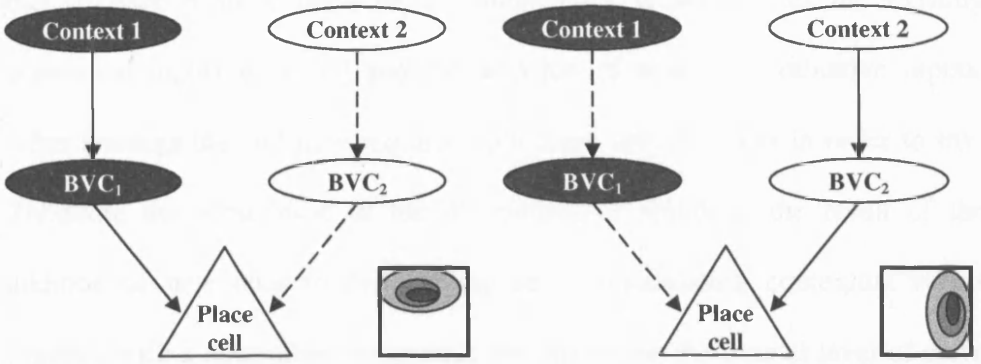


Figure 44. Possible explanation for why a place field shifts its field (remaps) in response to a context change (see Jeffery & Anderson, 2003). The cell in this example receives two sets of boundary inputs, one for each field, with each boundary input being controlled by a context input. Active elements (inputs) are depicted in black, solid lines, inactive elements are shown in dashed lines. Left – in context 1 (e.g. black box), the boundary input set activated by black drives place cell firing in the North-West corner of the box. Right – in context 2 (e.g. white box), the other boundary set is activated and the cell is driven to fire along the West wall.

The type of remapping seen with cells discriminating location is different to that seen with cells that have been previously observed to respond to geometric alterations (O'Keefe and Burgess, 1996). With O'Keefe & Burgess, deformations of box geometry led to place fields stretching or splitting, a pattern not seen here. As discussed above, place fields tended to switch on/ off or change preferred firing location. This pattern is commensurate with the kind of remapping seen following contextual changes. It is likely therefore that the acquired inputs (presumed to be inputs provided from distal cues

outside the box environment) functioned as additional context cues as opposed to geometric ones*. Additional support for this argument comes from the context specificity of the location discrimination. The discrimination shown was specific not just to a given cell but to a given field: a cell might discriminate when expressing one of its fields but not the other. In order for this to happen there must be a simultaneous weakening of the existing contextual inputs to a cell and the addition of new, discriminative inputs. After learning the cell now requires both these sets of inputs in order to fire. Therefore the acquisition of the discriminative ability is the result of the addition of new cues to the existing set of modulating contextual inputs together with a normalisation process that maintains the overall level of drive of the contextual inputs roughly constant.

*See Experiments 1 & 2 for a more detailed treatment of these points.

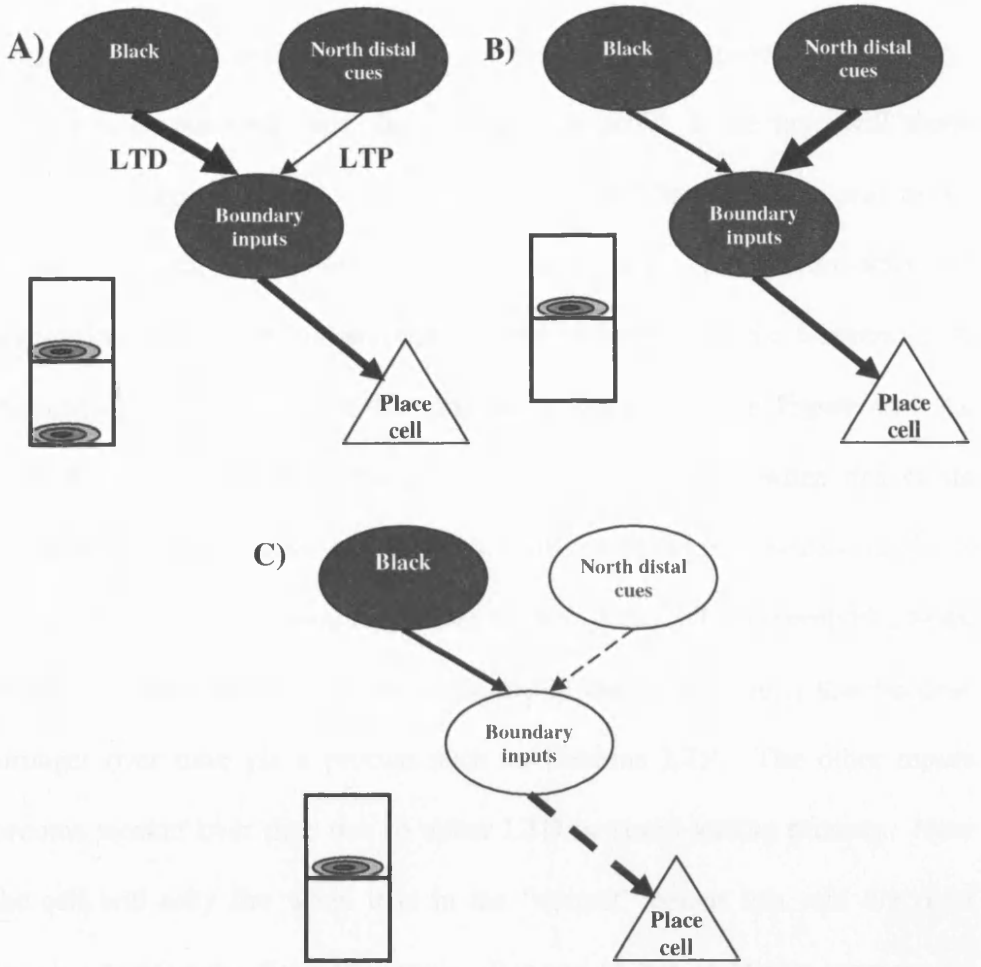


Figure 45. Hypothesized mechanism for the acquisition of location discrimination. This cell has a field that becomes specific to the North box. A) Initially the cell has the same field in the North and south locations as the context (black) dominates the boundary input. B) Over time the relative strengths of the context and discriminative cues (North distal cues) are altered so that the cell requires a conjunction of both context (black) and the discriminative cues to fire (via the boundary inputs). C) In the South location the North distal cues are not available and the cell doesn't fire. Active elements (inputs) are shown in solid, black lines, inactive elements in white, dotted lines. Thickness of a line indicates the relative strength of inputs. See text for further details.

Therefore in order for a discrimination to occur in the familiar environment two conditions must be met; a weakening of the existing contextual inputs and the presence of the correct distal cues. Because of this weakening, the correct

discriminative cues are necessary in order to drive the cell above threshold and cause the cell to express its field. Presumably this process is activity-dependent so that only those inputs that were active at the time will show synaptic strength changes. This indicates that the discrimination was never expressed in the novel environment because new, never-before-activated contextual inputs were initiated that had not undergone such a weakening. A hypothesized mechanism for this process is diagrammed in Figure 46. As with Figure 44, prior to learning, a cell expresses a field when one of its associated boundary input sets (BVCs) is driven by its associated contextual inputs. As well as receiving input from the BVCs the cell also receives a weak input from discriminative inputs (presumably the distal inputs) that become stronger over time via a process such as Hebbian LTP. The other inputs become weaker over time due to either LTD or some scaling process. Now the cell will only fire when it is in the "correct" colour box *and* the right location to drive the distal cue inputs. Because of this coactivity requirement the connections that go through other BVC sets do not become potentiated and the cell expresses non-discriminating fields in these contexts.

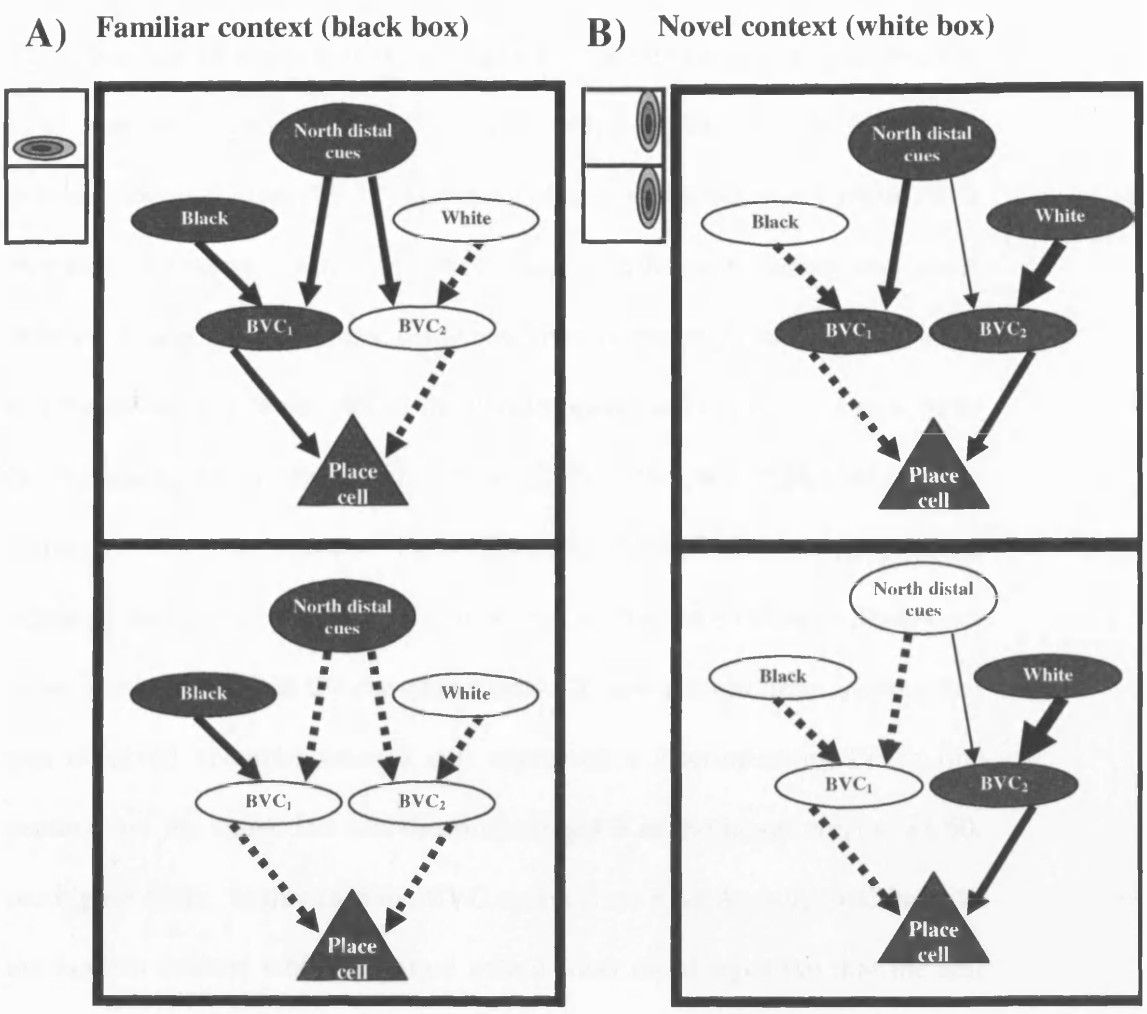


Figure 46. Hypothesized mechanism for the context-sensitivity of location discrimination, for a cell that discriminates in the familiar context (black here) and develops a non-discriminating field when the context is changed to white. The place fields are shown in the insets. Active elements (inputs) are shown in solid, black lines, inactive elements in white, dotted lines. Thickness of a line indicates the relative strength of inputs. (A) In the familiar context following learning the inputs have become altered so that the original contextual inputs are weak but the discriminative (distal) inputs have become stronger. In order to express a particular field both the correct contextual and distal cues need to be present. Therefore the cell only fires in the North box. (B) When the context is changed, a new set of contextual elements becomes active and these drive a second set of BVC inputs, thereby producing a new field. Because no activity-dependent changes have yet happened to these newly active inputs, the contextual inputs are still strong enough to drive the BVC inputs by themselves, so the cell expresses the same field in both the North and South locations.

The model can be applied to explain the four different types of remapping that were seen in this experiment (see Figure 47). In each of the four cases outlined above a given PF is expressed when a given boundary input set is recruited. Remapping reflects the activation of different boundary sets under different conditions. The most usual pattern of remapping observed was when a cell expressed different PFs in the two locations and expressed a new, non-discriminating PF in the novel context (25/50 cells; see Figure 47A). As mentioned above it is proposed that this occurs because the boundary inputs active in the familiar context come to depend on the discriminative distal cues being present as well as the contextual cues. A rarer pattern of remapping that was observed occurred when a cell expressed a discriminating PF in one context and the same, but non-discriminating PF in the novel context (3/50; see Figure 47B). In this case the BVC set receives a weak contextual input in the familiar context which is paired with a weak distal input (so that the cell exceeds threshold and fires). This results in a discriminating PF in the familiar context. In the novel context the BVC set receives a strong, unweakened contextual input with the outcome that the PFs in this context are non-discriminating.

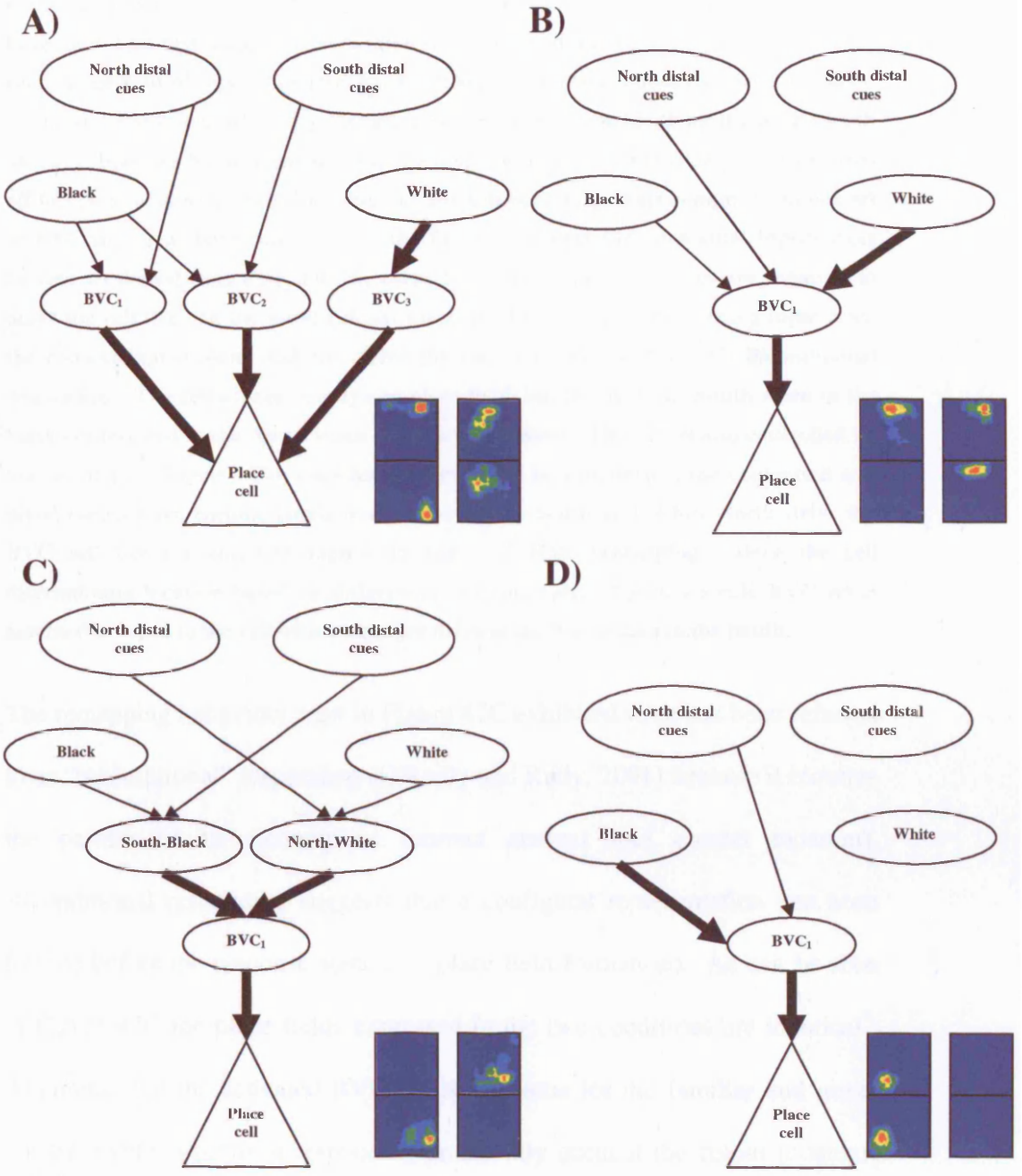


Figure 47. How the model can explain various types of remapping seen in the current experiment. A) The most commonly seen type of remapping with a cell that discriminated location in the familiar context by expressing two differently localized fields but with highly similar fields (but different to those in the familiar context) in the novel context. It is assumed that the cell receives three different sets of BVC inputs, each of which generates a distinct place field when activated. In the familiar context the contextual inputs (black) have become weakened by LTD. The cell will therefore only express this field when the box is in the black box and the North location. In the South

the cell expresses the second field, which needs both black and South inputs to be active. In the novel context the cell receives input from the third set of BVC inputs. Because no rearrangement of input strengths has yet taken place these inputs can drive the BVC inputs and result in a field being formed in either the North or South locations. B) A cell that discriminates North from South in the familiar context by switching one of its fields off but fails to do so in the novel context. The cell here only receives input from one set of BVC inputs in both contexts. In the familiar context the contextual inputs have become weakened such that both the correct contextual and distal cues are required to make the cell fire. In the novel context the same BVC set receives a strong input from the context inputs alone and can drive the cell in either location. C) Biconditional responding. The cell expresses only one place field, but this is in the South when in the black context and in the North when in the white context. This cell is also controlled by one set of BVC inputs. The response pattern could be explained if the contextual and distal inputs have become configured so that Black-South and White-North drive the BVC set, but the converse inputs do not. D) Rate remapping. Here the cell discriminates location based on differences in firing rate. Again, a single BVC set is assumed to input to the cell with a stronger drive in the North than in the South.

The remapping behaviour seen in Figure 47C exhibited what has been referred to as "biconditional" responding (O'Reilly and Rudy, 2001) because it requires the pairing of two conditions (correct context *and* correct location). Biconditional responding suggests that a configural representation has been formed before the response stage (i.e. place field formation). As can be seen in Figure 47C the place fields expressed in the two conditions are identical*. Assuming that the activated BVC set is the same for the familiar and novel conditions this pattern of responding could only occur if the colour (context) and location signals were bound together before arriving at the common BVC inputs.

The final type of remapping (rate remapping) occurred in only one of the animals. However, this did occur with several different cells (7/11) over

*It is, of course, possible that the PFs expressed in the two conditions happen to be similar just by chance. That is, the cell may have remapped its PF in both context/ locations and have remapped one of its fields to the same location as the first one.

repeated occasions (see Figure 47D and Figure 43). Here the fields in a given context were the same in both locations but displayed pronounced rate differences between locations. The cell is assumed to receive a set of BVC inputs that in turn receive a strong input from the North cues and a weak input from the South cues.

Paradoxically, this type of remapping has recently been seen in response to a contextual change (Leutgeb et al., 2005). Here changing the colour of the box environment whilst maintaining it in the same spatial location in the experimental room resulted in a rate remapping occurring. Recording from a box of the same dimensions and colour in a different room resulted in a complete remapping occurring where fields shifted their spatial locations, switched on/ off (Leutgeb et al., 2005). The interpretation of the data made by the authors was that rate remapping allows different events occurring in a given location to be discriminated. This seems counter to the findings given that in both that experiment and the current one the animal is experiencing the same "events" in both locations. Furthermore, recording from boxes of different colours in the same spatial location reliably resulted in rate remapping occurring (Leutgeb et al., 2005). As is evident from the data presented above this was not found here. Instead, following a change to the colour of the environment *in the same spatial location* as the previous one, the cells from three rats shifted their firing fields or switched on/off. The difference in results between the current experiment and Leutgeb et al., may be due to the different training regimes employed. Indeed with Leutgeb et al there is a rather large discrepancy between the number of pre-training days received in the different conditions – 14 for recordings made from boxes in

different rooms and 4 for the colour change in the same spatial location which may account for these differences.

6.4.2. Alternative models & explanations

As with previous work (Bostock et al., 1991;Jeffery, 2000;Lever et al., 2002b), the remapping seen in the familiar environment is reminiscent of an experience-dependent change in response to two highly similar stimuli. As elucidated above, a gradual change in the initially homogeneous representation suggests that some kind of plasticity is at work. This plasticity is not just a strengthening of the inputs to the BVCs but also must consist of a simultaneous weakening of some subset of the inputs to the BVC set. The second most common form of remapping seen in the current experiment was when a cell lost a field in one of the locations (within a given context). Attempts to explain this pattern using neural network models has revealed that Hebbian learning by itself is not sufficient to explain the loss of a field in one condition (Fuhs and Touretzky, 2000). Fuhs & Touretzky (2000) explored which learning rules would best account for this pattern. They found that neither simple Hebbian learning or a Hebbian covariance rule could explain these effects. A Hebbian covariance rule is one in which synapses are strengthened/ weakened according to how much the pre- or postsynaptic neuron deviates from its mean firing rate. Under this rule a neurons weights will change regardless of which environment it is active in. This results in the representations of previous environments being lost at the same rate at which new ones are acquired. The simulations run by Fuhs & Touretzky revealed that exposure to a second environment would destroy the representation of the first environment (Fuhs and Touretzky, 2000). This clearly cannot explain the

current results.

The BCM rule however successfully mirrored the results seen in Bostock et al., (1991). The results seen were not only contingent on the particular rule that was implemented but also on the architecture that the rule operated over. There are similarities between the model implemented by Fuhs & Touretzky and the BVC model. Both have inputs to place cells that are tuned to respond as Gaussian response curves; the Fuhs & Touretzky model has these attached to single point-like cues on the walls, the BVC model uses the whole wall. The Fuhs & Touretzky model uses a dual route architecture – information is passed from the entorhinal cortex (EC) (where the Gaussian tuned units tied to environmental cues reside) to CA3 either directly or via the dentate gyrus, which serves as a pattern separator (i.e. small changes in input equal large changes in output). The plasticity implemented via the BCM rule in Fuhs & Touretzky occurs at the EC to CA3 synapse (the perforant path input). With the BVC model plasticity would have to occur at the connection between the BVCs and place cells (as there are no other connections in the model). This could be equated with plasticity occurring between EC and CA3.

With the BCM rule, learning only occurs when both the pre- and postsynaptic neurons are active. Importantly, there is a sliding threshold which determines whether or not a connection between cells is potentiated or depressed. Potentiation occurs when the postsynaptic cell is strongly active, depression when it is weakly active. The sliding threshold means that even weak neural activity can result in a strengthening of synapses; conversely, when the system is in a high state of activity the threshold also increases so that not all neurons

can exceed it, resulting in synapses becoming weakened. In terms of place field activity this means that cells strongly active in one environment but weak(er) in a second one remain strong in the first but will become weaker (to the point of inactivity) in the second. Therefore the BCM rule can result in optimal pattern separation occurring.

As can be seen from Figure 43, the BCM rule may not be able to explain the pattern of remapping seen with cells that display pronounced differences in rate between two environments. If the model based on the BCM rule proferred by Fuhs & Touretzky holds true then the field with the lower firing rate (in this case in the South location) should become inactive, whilst the field with the higher firing rate should be constant. Instead over the course of 30 separate trials (15 in each location) over four days the field with the lower firing rate remains relatively constant and the field with the higher firing rate appears to become attenuated especially on the last day this cell was recorded on.

The process postulated in the model introduced above essentially proposes the opposite to what the BCM rule does. In the above model initially strong inputs become weakened and weak inputs strengthened. Because of this there must be a mechanism for normalising the total synaptic strength. As suggested above, synaptic scaling seems a plausible process by which this could occur. This allows the total activity of a neuron to remain stable within a given range whilst at the same time preserving the distribution of synaptic strengths.

Such scaling of synaptic inputs has been demonstrated in cultured cortical and

spinal networks and operates via increasing or decreasing *all* excitatory connections. The scaling process occurs over hours and days and is therefore commensurate with the time course of remapping seen in the current experiment. This can be contrasted with LTP which has much faster kinetics. LTP at hippocampal synapses typically involves NMDAR activation. Supporting evidence for the idea that the links between the inputs to place cells are LTP modifiable comes from studies that have manipulated the activity of NMDARs in CA1 (Kentros et al., 1998). In this study animals had the activity of their NMDARS blocked in CA1. Place cells successfully formed place fields following initial entry to a first environment, showing that NMDARs are not necessary for the formation of place fields. When exposed to this same environment, again under NMDAR blockade, the same place fields were expressed. However second entry to a novel environment (where the first occurred under NMDAR blockade) resulted in a new, previously unexpressed map being instantiated. It therefore appears that NMDARs are not required for place fields to form upon initial exposure to an environment; instead they seem necessary for the same map to be instantiated upon repeated exposures. Once the map has become stabilised then the processes of LTP/LTD allow synaptic scaling to take place.

7. General Discussion

Two main lines of enquiry have been followed during the course of this thesis; the influence of geometry and the influence of context on the firing of rat hippocampal place cells. Geometry was found to be important for the formation of coherent individual place fields and determining where in an environment they fire. Context was found to modulate the selection of which collection of place cells (place representation) was activated. As a result of these findings, a model of the putative inputs to place cells was formulated.

7.1. Geometric inputs to place cells

The first and second experiments sought to elaborate the influence of environmental geometry on place cell activity. The first experiment attempted to assess the importance of extended boundaries on the formation and maintenance of place fields. To this end, a novel experimental setup was arranged whereby place cells were recorded as animals moved around an environment that was as close to "boundary-less" as was physically possible. Initially cells were recorded in a closed configuration i.e. with all the walls present, thus constraining the animal within its boundaries. Following this session a single wall was removed from the box, therefore allowing the animal access to the rest of the room. In order to prevent excursions into the parts of the room closest to the walls, a sound barrier was created which prevented animals accessing this part of the room. By gradually removing boundaries from the box the nature of the geometric input to individual place cells was investigated.

The main findings from these manipulations was that place fields fired over a

larger area of the environment and looked less and less like "normal" place fields recorded in the closed configuration of the box. As well as an increase in firing area, place fields also became less coherent. Furthermore, the trial-to-trial stability of place fields was affected. On several occasions the box environment was maintained in a 3-wall configuration and moved small amounts around the experimental room (approximately ½ the width of one of the box walls). Despite these small shifts in box position, and the fact that the walls/pillars were the only stable features of the environment, place fields were reliably different from trial to trial. Thus, following sequential boundary removal, place fields underwent a complete remapping from trial to trial. Unsurprisingly, day-to-day stability was also very low. Before the main experimental trials were run, a screening procedure was conducted (this was not entered into the analysis) in a different experimental room. Cells recorded in that environment (which was rich in terms of distal cues as the room was fully lit and similar to the room used in Experiment 3) *were* stable from day to day, further reinforcing the idea that the impoverished environment in the main experimental room could have been responsible for the breakdown in place fields.

The results from this experiment were analysed in four different ways that attempted to account for the different reference frames to which the place cells could have organised their responses. These responses included,

i. place fields remaining strongly driven by the boundaries of the box environment and maintaining good place fields within those boundaries

ii. place fields remaining anchored to the experimental room cues (or some uncontrolled cues) and firing in terms of the coordinate system described by the room

iii. a breakdown in the control of place fields by the boundaries following boundary removal and firing primarily outside the region described by those boundaries

iv. a hybrid mixture of these three possibilities.

However, these analyses all led to the same conclusion: place cells fired over a larger portion of the environment and became less coherent following boundary removal. Despite this, it was found that cells *could* form place fields even on trials where there was only a single pillar. In terms of the model proposed in Experiment 3 this suggests that boundaries can drive place cells above threshold but that they are not sufficient to maintain fields across trials. Initially, the modulation of context does not appear to be important here. Arguably the context is the same from trial to trial; the rat is after all in the same room with the lights off and is performing the same behavioural task. As proposed in the discussion for Experiment 3 it is also a possibility that the geometric arrangement of the environment can be fed back into the hippocampus as contextual information. When the geometry of the environment is repeatedly altered this information could come to be represented as a contextual change. Place cells would reflect such a change by exhibiting a complete remapping as described above.

Another distinct possibility is that the experience of moving around outside the box in some way reduces the salience of the boundaries. It is possible that

once the animal has full access to the boundaries and can move around them from all sides they come to be perceived not as boundaries but more as discrete objects. As has been seen with other studies (Cressant et al., 1997) objects placed at the centre of an environment are not able to control place cell firing with the same efficacy that objects placed at the periphery can. If such effects were occurring based on the animals experience outside the local box environment, then one prediction is that changes in cell activity might be seen when all the walls are replaced and the box is again in a closed configuration. Such a change could include a remapping from the place fields that were seen on the initial exposure to the closed box at the start of the session. On several occasions exactly such a manipulation was performed. It was found that place fields were established as with the initial closed trial and that no remapped fields were observed i.e. that place fields seen in the initial closed box session and the final closed box session were the same. This is a result that is further supported by the findings from Experiment 3.

Interpreting these results in terms of the BVC model suggests that as walls were gradually removed from the environment the boundary vector inputs to place cells were reorganised. This reorganisation meant that following each wall removal manipulation the set of BVCs that specified where the cell should fire were selected anew. This resulted in the observed lack of stability from trial-to-trial and day-to-day.

With Experiment 2 less dramatic alterations were made to an environment similar to that used in Experiment 1. Instead of wholly removing boundaries, an individual wall was moved apart from the local box environment enough to

allow the rat access to the rest of the experimental room. The nature of the inputs to individual place cells was elaborated in further detail. The results from this experiment were that place cells exhibited changes in their firing fields suggesting they were responding to a subset of environmental boundaries. Roughly a third of cells displayed pronounced differences in rates between the bounded and unbounded environments, another third had fields that broke down over time in the unbounded environment and the final third showed no change between the two environments. Plastic changes to place fields were also seen, reminiscent of those seen with Lever et al., (2002). This suggests that experience-dependent modifications were occurring to the place fields. The "standard" BVC model is not able to account for these changes without the modification that the connections between BVCs and place cells are able to undergo dynamic alteration.

The rate-remapping cells seen in Experiment 2 imply that the Gaussian tuning curves of different BVCs are being brought into alignment. This results in an increase in the cells firing rate over the trial. Interestingly the rate always increased in the unbounded condition compared to the bounded condition, a finding well replicated in the modified BVC model proposed by (Barry et al., 2006).

7.2. Context inputs to place cells

The second experiment provides more evidence that plasticity can occur at the level of the geometric inputs to place cells. Instead of the dramatic change made in Experiment 1 a much smaller change was made to the experimental environment. Rather than wholly removing a boundary, a single wall was

separated and moved away from the rest of the box. Whilst providing the animal access to the rest of the experimental room this manipulation still preserves the presence and inter-relations of the four most immediate boundaries. As with the previous experiment, the path of the animal was constrained by the activation of a sound barrier. Alternating trials were run between the closed and open box configurations. Place fields responded in one of several different ways when a wall was moved away from the rest of the box. Some place fields were unchanged, exhibiting the same place fields in the open box configuration as in the closed box. These cells displayed the same firing rate in both types of trial and can be distinguished from a second group of cells that, whilst showing the same place field in both situations, nonetheless also had consistently different firing rates in the two environments. Yet other cells became unstable when the wall was moved. These cells had stable, persistent fields in the closed box arrangement yet when a wall was moved away from the box they lost spatial specificity and fired over large parts of the environment. Note that the converse of this process was not observed; poorly specified fields were never seen in the closed box configuration that subsequently became stable and persistent in the open configuration.

Perhaps the most interesting finding was the observation of gradual remapping occurring in the closed box following repeated exposure to the open box configuration. Here one of two responses was seen to occur. The first was the detachment of a field from one wall and it subsequent attachment to an orthogonal wall. The other form of remapping occurred whereby a field was seen to develop a subfield (that had a lower peak rate than the primary field)

that, over the course of several trials, became the stronger field with the primary field becoming a subfield. Such a result suggests that some experience dependent alteration is occurring to the place field which is causing it to change its properties in the closed box configuration.

The final experiment was motivated by two different, but related, findings. The first was suggested by Experiment 1 and Experiment 2. An implication from these experiments is that geometric changes to an environment can come to be construed as contextual changes, and furthermore that there is possibly a learnt element to this process. The final experiment therefore sought to investigate more purely the influence of context by holding the geometry of an environment constant whilst manipulating the context alone.

The other impetus to the final experiment was from a finding by Jeffery (2000) that place cells could gradually come to differentiate between two neighbouring locations. Such discrimination developed over time following repeated exposures to the same environmental stimuli. The stimuli immediately available to the animal (local cues) were ostensibly identical in both locations; the only systematic difference between the two locations was the view of the distal room cues available from each place. Therefore, place cells that were able to differentiate between the two locations did so based on the distal information.

The purpose of Experiment 3 was to reproduce this basic result and extend it by examining the nature of the learnt discrimination. It was therefore of interest to examine the consequences of introducing a contextual change to an environment that had been differentiated as described above. Cells that

differentiated the environment before the contextual change was introduced did so by exhibiting a remapping. When cells differentiated between the two neighbouring locations the type of remapping observed was what has been called complete; place fields became active or inactive, or radically changed their preferred firing locations.

Once such a remapping was observed the context of the environment was altered by changing the colour of the walls and floor. Such a change to the environment is also known to induce a complete remapping (Anderson and Jeffery, 2003;Bostock et al., 1991). Several outcomes following this change were thought possible. First, place cells could have continued to discriminate the new context as they had in the old context. Second it was possible that only those cells that were active in the old context would differentiate the new one i.e. some kind of activity-dependent learning had occurred. Last, cells in the new context could fail to differentiate the two locations at all. The last outcome was the one that occurred, leading to an extension of the boundary vector cell (BVC) model of place cell activity being proposed.

Interestingly, the manner in which the discrimination of the two familiar locations occurred differed between different animals. Two of the animals remapped the familiar context by primarily shifting their fields to novel locations. A third animal remapped mostly by switching cells on/off and the fourth animal had cells that remapped by consistent changes in their firing rates. It should be noted that the pattern of rate remapping could possibly be a less extreme form of the on/off behaviour of cells seen in the third animal. Despite these inter-animal differences, and the rapidity with which one animal

remapped the novel context, the overall result remains the same; animals were unable to immediately transfer the discrimination acquired in one context to a new, never-before experienced one.

7.3. Model of geometric and contextual inputs to place cells

The model begins with the proposition that place cells receive inputs from cells that are tuned to respond to the presence of extended surfaces or boundaries. The geometric inputs are equivalent to the boundary vector inputs described by Hartley et al. (2000), and are responsible for selecting which particular field is expressed by the cell. The geometric inputs respond at a given allocentric bearing and direction to a barrier. The thresholded and summed input of several BVCs to a place cell causes it to form its place field. By determining the subset of BVCs which best fit a set of experimental data it is possible to use the model in this form to predict what will happen to a place field when an animal is introduced into a geometrically different environment. However, this formulation the model is relatively static as it incorporates only hard-wired, feed-forward connections from BVCs to place cells. Concordantly, there is no account of activity-dependent changes that can occur during/following learning. The results from Experiment 2 suggest that after exposure to geometrically similar, but altered environment, plastic changes can be observed that cannot be accounted by the model in this form.

In order to account for these observations the connections from BVCs to place cells need to be able to undergo change. Indeed such a formulation of the BVC model has been recently proposed based in part on the experiments described here (Barry et al., 2006). In this re-formulation of the model the

BCM rule is used to update the weights between BVCs and place cells. The BCM rule can overcome some of the difficulties present in a straightforward Hebbian-based rule. Unlike the Hebbian learning rules, the BCM rule allows both positive and negative weight changes. With sustained activity of a postsynaptic cell above a threshold an increase in the efficacy of synapses onto that cell occurs. Activity below that threshold results in a decrease in efficacy. Importantly, the level of this threshold is dynamic and determined by the activity history of the postsynaptic cell. A highly active postsynaptic cell will shift the threshold higher thus making it more likely that a decrease in efficacy will occur, with the converse happening for a weakly active cell. Furthermore, weight changes only occur if both the pre- and postsynaptic cells are active, as occurs under normal, homosynaptic plasticity. The BCM rule has also been employed by Fuhs & Touretzky (2000) and was found to be more capable of accounting for the experimental data they examined than other rules such as Hebbian-based learning rules (Fuhs and Touretzky, 2000). The altered form of the BVC model proposed by (Barry et al., 2006) was able to account for the experimental data presented here reasonably well, with only minor differences.

Unless the altered form of the BVC model also makes additional assumptions that BVCs can also respond to the colour of walls, it is unable to account for the data presented in Experiment 3. In order to explain this data, an additional layer of units was added to the model. These units impinge on *sets* of BVCs and modulate which set of BVCs is selected. Therefore, a hierarchical relationship is assumed to exist between the geometric and contextual inputs. Simply, the contextual inputs are responsible for selecting which geometric

inputs are expressed by the cells in a particular environment. At a broad level these two types of input can be seen to be spatial (geometric) and non-spatial (contextual). There is accumulating evidence that the main afferents to the hippocampus send both types of information (Fyhn et al., 2004;Hargreaves et al., 2005). In light of this it is proposed that the role of the place cells in CA1 is to instantiate a representation that allows accurate representation of an animal's location in a specific context. This representation can be updated following changes in context as the context inputs modulate the activity of sets of BVCs, without changes occurring to explicitly spatial/ geometric variables. This implies that the activity of CA1 place cells reflects not just a map of space but rather a map of spatial context.

As mentioned above, learning also plays an important role in the model; it is only with repeated experience of the two neighbouring locations that cells come to discriminate them. The pattern of remapping seen in Experiment 3 was complete – cells switched on or off or shifted their firing locations to unpredictable locations. As the only way to discriminate the two locations was the relative locations of the distal cues these must have played an important role. The two ways they could have caused a remapping are, i) they served an inhibitory role causing cells to switch off or, ii) they served an excitatory role with the overall level of excitation maintained via synaptic scaling mechanisms. For reasons espoused earlier it appears that the second scenario is the most likely one. This is a conclusion further supported by the following argument. The discrimination was specific to a given field and not to the whole cell; a cell could express place fields that differentiated the old context and a different field that did not discriminate the new context.

Therefore the contextual inputs functioned to switch on/off a field rather than switch on/off a cell. For this to occur there must have been a simultaneous weakening of the pre-existing contextual inputs to the cell as well as the addition of new discriminative inputs. Following learning the cell requires both of these inputs to fire. Therefore expression of the discriminative ability occurs when new cues are added to the set of contextual inputs. Critically the cell cannot exceed threshold with only one of these two cues present – the correct discriminative cues are required to drive the cell to express its field. In concert with a normalization process that scales the overall activity of the cell, this conceptual model can explain how such discrimination is acquired.

The above framework can also account for the failure of place cells to differentiate the new context. In the novel context new contextual inputs are activated that have never undergone such weakening. Because of this the cell will express the same field whether or not the distal cues are present. Activity-dependent learning in the old context means that the learning only occurs on those collections of BVCs that are active at the time, possibly by a mechanism similar to the BCM rule elucidated above. As with Hebbian-based learning mechanisms and the BCM rule, connections that are routed through other BVCs are not potentiated. Introduction to a new, never-before experienced context results in the activation of BVCs that have never undergone such learning. As a result place cells express similar fields in both locations in the new context.

Based on these arguments the following scenario can be constructed. When an animal enters a new environment a random set of place cells becomes

active, with each cell having the requisite inputs to specify a place field. Supporting evidence for this comes from experiments that have manipulated synaptic plasticity either by interfering with protein synthesis (Agnihotri et al., 2004) or by electrically interfering with the inputs to place cells (Dragoi et al., 2003). Here, although the presence or absence of fields is affected, the initial existence and morphology are normal. From this it can be concluded that, at least initially, activity-dependent synaptic plasticity is not required for the rapid establishment of place fields. Coactivity of the now active place cells with the contextual cues specific to that environment causes a strengthening in the connection between the boundary inputs and the contextual inputs. That such a strengthening occurs is supported by the results seen in Experiment 3 and in other studies (Bostock et al., 1991;Jeffery, 2000;Lever et al., 2002b). These results suggest that the information that distinguishes the environments comes to assume a relevance it did not previously possess. The nature of these connections is presumably NMDAR-dependent as evidenced by the Kentros et al., (1998) result that NMDAR blockade abolishes place field stability for more than a few hours.

As well as a strengthening between the active geometric inputs and the contextual inputs, there must also be a simultaneous weakening between the contextual inputs and the other inactive (or weakly active) geometric inputs, such that the contextual cues only elicit one set of geometric inputs on subsequent reintroductions to the same environment. A similar weakening could also be occurring between the inactive contextual inputs and the geometric inputs so that the cell only comes to be driven by the relevant contextual cues. A mechanism underlying these weakening processes could

be heterosynaptic LTD – this is also assumed to be dependent on NMDARs as LTD in the hippocampus is dependent on these receptors (Liu et al., 2004).

7.4. Conclusion

The work described in this thesis was in part informed by the predictions of a specific model of place field formation and responding, the Boundary Vector Cell (BVC) model (Hartley et al., 2000;O'Keefe and Burgess, 1996). The findings from the first two experiments largely support the BVC model. Moreover, partly as a result of this work the model has recently been extended to include the influence of plasticity on the BVC-to-place cell connection (Barry et al., 2006). A novel result was also seen with the rate remapping cells seen in both Experiment 1 and 2, although the finding does not support recent conclusions made about the likely nature of rate remapping (Leutgeb et al., 2005). The model proposed based on the work carried out here integrates the influence of both geometric and contextual cues and seeks to explain the inter-relationship between this important sources of information for hippocampal place cells.

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