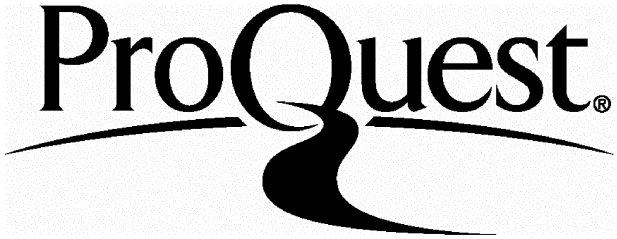
STUDIES ON DIRECTIONAL SIGNALLING IN THE HIPPOCAMPAL FORMATION OF THE RAT

Francesca Cacucci

Thesis submitted for consideration for the degree of Doctor of Philosophy

Department of Anatomy and Developmental Biology
University College London
February, 2003



List of figures

The figure directly follows the page listed.

List of tables

The table directly follows the page listed.

ABSTRACT

This thesis examines the properties of a new category of cell in the rodent hippocampal formation: topodirectional (TD) cells. Cell activity was extracellularly recorded in freely moving rats. TD cell firing is modulated by three variables: the animal's allocentric position in the environment, its head direction in the horizontal plane, and the theta oscillation in the hippocampal formation. Thus TD cells combine properties of well characterised cell types within the hippocampal formation: place cells, head direction cells and theta cells.

The thesis begins with a general introduction to the anatomy and physiology of the hippocampal-parahippocampal network. The methods section follows, with the detailed description of the recording protocol and analytical procedures.

The results section opens with the histological findings, regarding the anatomical location of TD cells, and shows that TD cells were mostly found in the dorsal extent of presubiculum and parasubiculum. The extracellularly acquired waveforms were analysed: TD cells show shorter duration and smaller amplitude waveforms than head direction cells. Using several quantitative measures of locational and directional spatiality, TD cells were then compared to place cells and head direction cells. The directional signal of TD cells was found to be stronger and more robust than their locational signal.

TD firing is strongly modulated by the theta oscillation, more so than that of place cells. The depth of theta modulation in TD cells is similar to that reported for cells in the septum, which are thought to drive hippocampal theta oscillation. TD cells preferentially fire around the trough of the locally recorded theta.

TD cells were recorded in four environments. Environmental geometry selectively affected the locational properties of TD cells, while leaving directional properties unchanged. The changes in the locational properties were similar to those reported for place cells. TD cells were also studied under other environmental manipulations, including darkness.

Discussion focuses on the particular contribution of TD cells to the hippocampal navigational system. It provides hypotheses of how the allocentric locational and directional properties of TD cells emerge from sensory and idiothetic information. It also suggests that TD cells may represent inputs to the hippocampal place cells.

Introduction

Chapter I

The anatomy of the hippocampal formation and the parahippocampal region.

Nomenclature

Throughout this brief anatomical description I will follow the nomenclature proposed by Scharfman et al. (2000) derived from a group discussion at a meeting focused on the anatomy and functional role of the parahippocampal region.

According to this nomenclature, the hippocampal formation includes the dentate gyrus (DG), area CA3/CA1 and the subiculum while the parahippocampal region comprises the pre- and parasubiculum, the entorhinal cortex (EC) and the peri- and postrhinal cortices (the latter is considered to be homologous to the parahippocampal cortex in primates including humans).

The segregation of these structures into a hippocampal formation and a parahippocampal region is based on differences in lamination and connectivity. The hippocampal formation shows a trilaminar organisation which defines the allocortex (as opposed to the neocortex, also known as isocortex, which comprises six layers). The parahippocampal region comprises cortical areas which represent the transition from allocortical to neocortical organisation.

The border between the subiculum and the presubiculum, which defines that between the hippocampal formation and the parahippocampal region, is marked by the appearance of a new cortical sheet. This sheet consists of the superficial layers of pre- and parasubiculum which are separated from their deep layers by a cell-free zone, the lamina dissecans. The deep layers of the pre- and parasubicular cortices appear to be the continuation of the subicular sheet (see fig I.2). This peculiar cytoarchitectonical organisation of pre-, parasubiculum and entorhinal cortex into superficial layers separated from the deep layers by the lamina dissecans defines the periallocortex. The perirhinal and postrhinal cortices are referred to as proisocortex and are characterised by the disappearance of the lamina dissecans. The overall lamination of the proisocortex is very similar to the one found in neocortex, the only difference being that peri- and postrhinal cortices lack a marked granular cell layer IV.

Hippocampal connectivity differs from that of neocortex by lacking much of the strong, reciprocal innervation seen in, for instance, the visual cortex (ie between any given cortical areas the strength of the forward and backward projections is usually comparable). It is much simpler, and more serial, but we will see that the classical

view of the flow of information through the tri-synaptic loop is an oversimplification, and that the hippocampal connectivity should be viewed more as a series of parallel circuits.

The parahippocampal region shows a more "neocortical" pattern of connectivity, including at least two sets of reciprocal connections, one between peri- and postrhinal cortices and entorhinal cortex, the other between pre- and parasubiculum and entorhinal cortex.

Gross morphology

The hippocampal formation is a rather large structure, representing about half the cortical volume of the rat's brain. Figure I.1 is a sketch depicting where the hippocampus sits in the rodent brain. Its long axis extends from the septal nuclei of the basal forebrain rostrally and bends caudo-ventrally into a cashew-nut shape, reaching the temporal lobe.

The long axis of the hippocampus is referred to as septo-temporal axis, and orthogonal to this, running medial to lateral, is the tranverse axis. These two axes represent important landmarks in the structural connectivity of the hippocampal formation.

The horizontal section in figure I.2, through the hippocampal formation and the parahippocampal region reveals the shape of its basic components. The darkly stained material that defines two interlocked, V-shaped structures represents the granule cell layer of the dentate gyrus (DG), and the pyramidal layers of fields CA1-3 (Cornu Ammonis). This section illustrates the trilaminar organisation of the structures which make up the hippocampal formation (DG, CA1, CA3 and subiculum), and shows the emergence of new layers at the border between subiculum and presubiculum and also how the deep layers of pre- and parasubiculum appear to be a continuation of the pyramidal layer of the subiculum. At the transition point between entorhinal and perirhinal cortices a new, subtler, change in lamination occurs, with the disappearance of the lamina dissecans.

Figure I.3 is a drawing of the left hemisphere of the rat brain seen from a caudo-lateral point of view to highlight the position of the medial and lateral portions of the entorhinal cortex (MEA and LEA respectively), the perirhinal cortex just dorsal of the rhinal sulcus and the post-rhinal cortex which is situated dorsocaudally to the MEA.

General connectivity

The diagram in fig I.4 illustrates the major connections of the parahippocampal-hippocampal regions. Cortical afferents reach the hippocampal formation through the entorhinal cortex (both directly or channelled through the rhinal cortices).

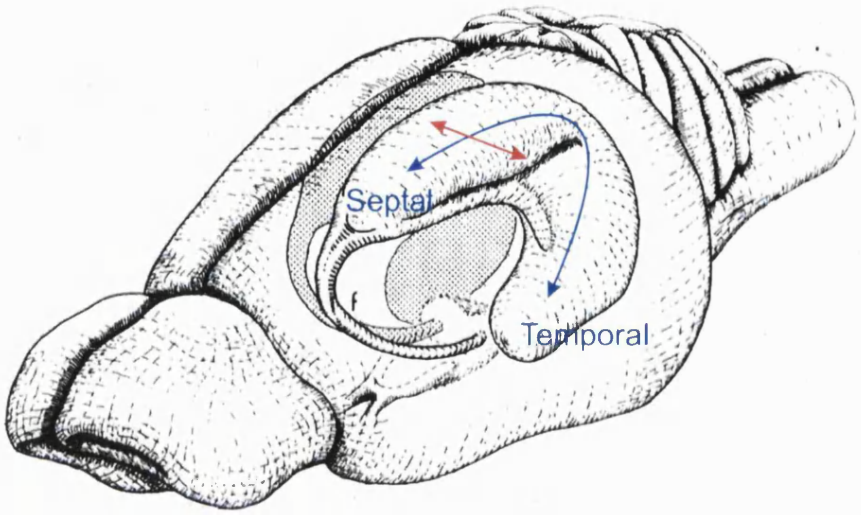


Fig I.1. Drawing showing the position of the hippocampal formation within the rat brain. The septo-temporal axis (blue) runs between the poles of the hippocampal formation marked septal and temporal. The transverse axis (red) runs medial to lateral. (Adapted from Amaral and Witter, 1995).

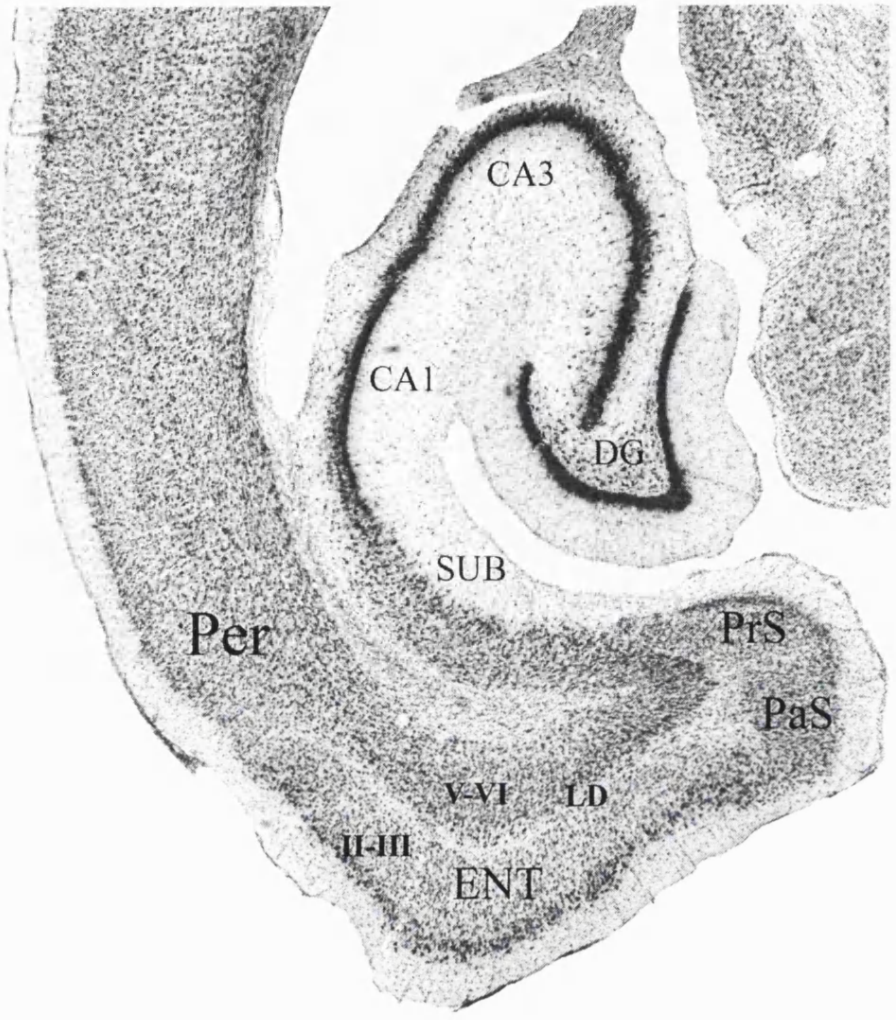


Fig I.2. Horizontal section through hippocampal formation and parahippocampal region of the rat brain. The trilaminar structures that compose the hippocampal formation are the Dentate Gyrus (DG), CA1-3 pyramidal cell layers, and the subiculum (SUB). The parahippocampal region is composed of the Presubiculum (PrS), Parasubiculum (PaS), Entorhinal cortex (ENT) and Perirhinal cortex (Per). The transition to the parahippocampal region is marked by an abrupt increase in the number of layers. The newly appearing layers form the superficial parts of these structures (II-III), while the deep layers (V-VI) are a continuation of the Subiculum. The transition between the Entorhinal and Perirhinal layers is marked by the disappearance of the cell-free zone, lamina dissecans (LD).

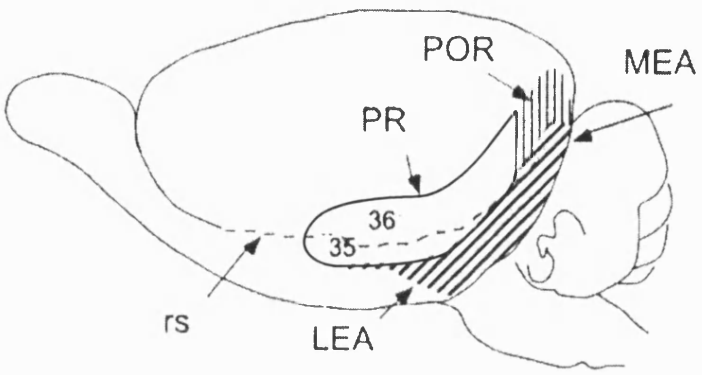


Fig I.3. Diagram of left hemisphere of rat brain, showing positions of medial and lateral areas of the entorhinal cortex (MEA, LEA), postrhinal cortex (POR), perirhinal cortex (PR, areas 35 and 36) and rhinal sulcus (rs). (Adapted from Burwell, 2000).

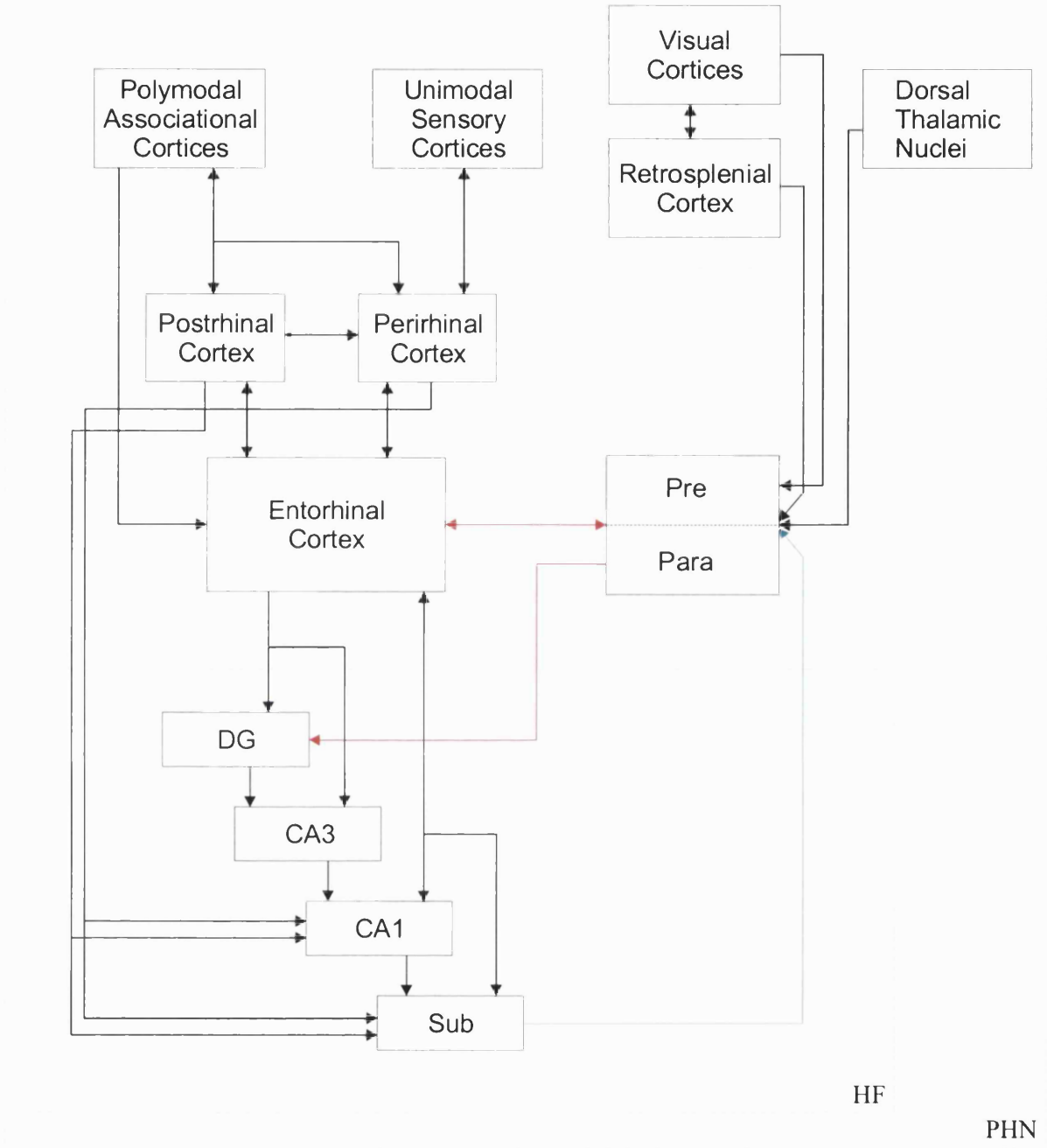


Fig I.4. Diagram showing the major connections of the hippocampal-parahippocampal regions, with particular emphasis on the pre and parasubiculum. The grey dashed boxes demarcate the hippocampal formation (HF) and the parahippocampal network (PHN). The pre- and parasubiculum both project to (Dentate Gyrus; via Entorhinal cortex , red) and receive projections from (Subiculum, blue) the hippocampal formation. The pre- and parasubiculum also channel information from Dorsal Thalamic Nuclei into the Hippocampal Formation, via the Entorhinal Cortex.

Some subcortical afferents directly innervate all fields of the hippocampal formation (septum, basal nucleus of the amygdala, midline thalamic nuclei, hypothalamic nuclei, brainstem and other aminergic nuclei), while others (dorsal thalamic nuclei, eg anterodorsal, anteroventral and laterodorsal nuclei) are channelled through pre- and parasubicular cortices, via the entorhinal cortex.

The entorhinal cortex gives rise to the "perforant pathway" to the hippocampal formation, which can be subdivided into two streams:

1) one originating from layer II of entorhinal cortex and innervating DG and CA3;
2) the second originating from layer III of entorhinal cortex and targeting CA1 and subiculum.

The main intrinsic connections of the hippocampal formation are represented by:

1) mossy fiber projection from Dentate gyrus to CA3;
2) CA3 to CA3 recurrent collaterals;
3) Schaffer collaterals, from CA3 to CA1;
4) Projections from CA1 to subiculum.

The organisation of the perforant pathway, combined with the layout of the hippocampal intrinsic connections suggests that both serial and parallel information processing take place within the hippocampal formation.

The subiculum is the major output region of the hippocampal formation. It projects to a number of cortical and subcortical regions, and, in the parahippocampal region, to the deep layers of entorhinal cortex, perirhinal cortex, pre- and parasubiculum.

The CA1 field also projects to deep layers of entorhinal cortex, perirhinal cortex, and, if not as strongly as the subiculum, to a number of cortical and subcortical regions.
Information processed within the hippocampal formation is thus relayed to the deep layers of entorhinal cortex (from CA1 and subiculum). From there, it can be either fed back to the hippocampus, by way of the associational connections from deep to superficial layers of entorhinal cortex, or channelled to the cortical mantle, directly (from a very restricted area of entorhinal cortex) or through peri- and postrhinal cortices, or channelled to other subcortical sites (ie thalamus, mammillary nuclei).

Connectional organisation along the septo-temporal axis

Because the entorhinal cortex is the major relay for incoming sensory information in the hippocampal formation, the topographical organisation of the perforant pathway connections has strong functional implications for the kind of processing that will take place in the hippocampus.

The EC can be subdivided into three longitudinal strips (see fig I.5), each projecting to a different septo-temporal (or dorso-ventral in rat) level of the hippocampal formation (each of these including both cytoarchitectonic subdivisions of the EC, the medial and the lateral entorhinal areas which will be discussed in more depth later; Dolorfo and Amaral, 1998a; Witter, 1989; Ruth et al, 1982; Ruth et al, 1988). The most lateral and caudomedial portions of the EC, which receive major inputs from the adjacent perirhinal and postrhinal cortices, project predominantly to the septal third of the hippocampal formation. These include prominent olfactory, auditory, visual and visuo-spatial inputs. The most rostromedial portions of the EC, which receive prominent inputs from the limbic and periamygdaloid cortices, project more temporally in the hippocampal formation. The intermediate zone of the EC (midmediolateral portions of the EC) projects to the intermediate parts of the hippocampal fields. It is also interesting that the organisation of the entorhinal intrinsic connectivity preserves the segregation of the three longitudinal strips (Witter, 1989; Kohler, 1986; Kohler, 1998; Dolorfo and Amaral 1998b).

Within the hippocampal formation, the projections from DG to CA3, CA1 to subiculum and subiculum back to entorhinal cortex, show a similar topographical organisation into three septo-temporal strips (Amaral and Witter, 1995). However, the CA3-CA3 auto-associative connections (Tamamaki et al, 1984, 1988), and the Schaffer collaterals (CA3 to CA1 connection; Li et al, 1994) show a highly divergent organisation, not preserving the septo-temporal organisation outlined above.

The pattern of connectivity just described, suggests that septal levels (dorsal) of the hippocampal formation most likely process sensory-related information whereas the temporal hippocampus is more involved in visceral-related processing (Witter et al, 2000). Both behavioural and electrophysiological studies are beginning to demonstrate functional differences along the septotemporal hippocampal axis. Behaviourally, it has been shown that rats with lesions restricted to the septal extent of the hippocampus exhibit longer escape latencies in the Morris Water Maze task than rats with lesions restricted to the temporal portion of the hippocampus (Moser et al, 1993). A more recent paper (Bannerman et al, 2002) showed that ventral hippocampal lesions had no effect on T-maze alternation and on a working memory version of the Water Maze, even when task difficulty was increased by introducing delays. Moreover, place cells recorded from dorsal hippocampus show place fields with higher spatial selectivity than place cells recorded from more temporal portions of the hippocampus (see chapter IV, p.35, Jung et al, 1994).

Connectional organisation along the transverse axis

We have just described how input modality within the hippocampal formation is not homogeneous along the septo-temporal axis. Another feature of hippocampal connectivity is that different kinds of processing occur at different transverse levels of CA1 and subiculum. This is due to the peculiar connectivity pattern of the perforant pathway connections to these hippocampal fields.

We have already mentioned that the perforant pathway can be subdivided into two components: one originating from cells in layer II of EC which innervates DG

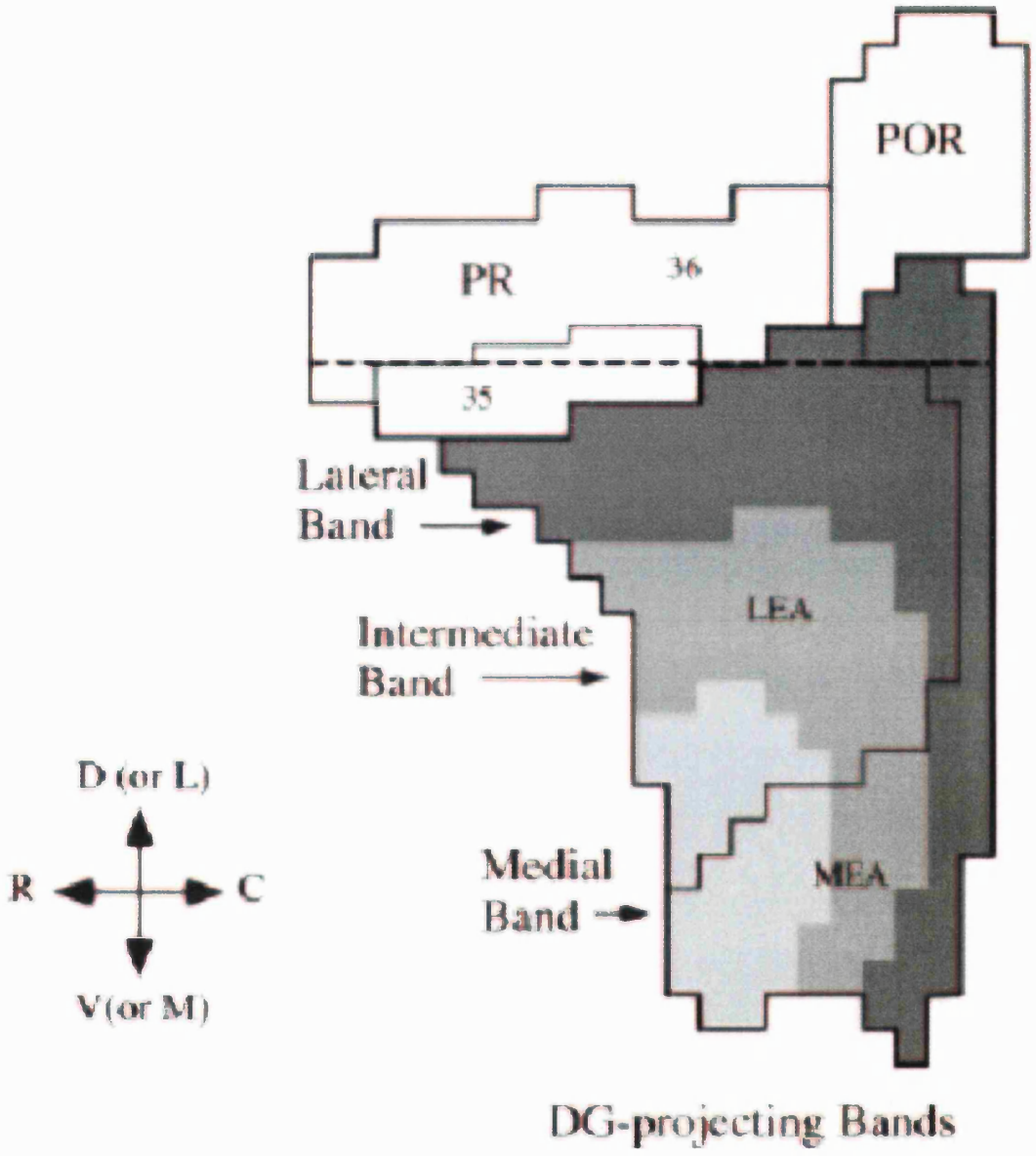


Fig I.5. Diagram showing an unfolded map of the perirhinal (PR; areas 35 and 36), postrhinal (POR) and entorhinal (EC: lateral entorhinal area [LEA] and medial entorhinal area [MEA]) cortices, showing the approximate location of the lateral, intermediate and medial dentate-projecting bands in the entorhinal cortex. Note that each band includes portions of both LEA and MEA. Adapted from Burwell and Amaral (1998a).

and CA3, the other originating from cells in layer III of EC innervating CA1 and subiculum (Steward and Scoville, 1976; Witter and Amaral, 1991). A further level of complexity is added by the fact that the perforant pathway comprises two components, orthogonal to the ones just described, which originate from two cytoarchitactonically defined subdivisions of the EC. These subdivisions have been referred to as the lateral and medial entorhinal areas (LEA and MEA; fig I.3). The lateral and medial perforant pathways carry different information and show distinct terminal distributions in their respective hippocampal targets, depending on whether they originate in layer II or III. Projections from cells in layer II discriminate along the radial axis of DG and CA3, i.e. cells in layer II of LEA project to the outer one-third of the molecular layer/stratum lacunocum-molecolare of the DG and CA3, while layer II cells of MEA project to the middle one third of these layers. However, they do not discriminate between transverse levels. Therefore lateral and medial layer II components likely influence the same cells in the DG and CA3 (McNaughton and Barnes, 1977). Unlike them, the lateral and medial layer III components show an entirely different, non-overlapping terminal distribution in CA1 and the subiculum along the transverse axis. Projections from layer III of the LEA terminate in the distal part of CA1 and the proximal part of the subiculum (at the border between CA1 and subiculum). In contrast, fibers originating in layer III of the MEA, terminate in the proximal part of CA1 and the distal part of the subiculum (Witter et al, 2000). If we add that projections from CA1 to the subiculum (Tamamaki et al, 1987; Tamamaki and Nojyo, 1990, Amaral et al., 1991) and from CA1 and subiculum back to entorhinal cortex (Tamamaki and Nojyo, 1995) preserve this columnar organisation, we realise that while at the level of DG and CA3 there is a convergence of the medial and lateral entorhinal inputs, at the level of CA1 and subiculum, two parallel channels of information exist.

This organisation is likely to have functional value, because inputs channeled through the medial and lateral portions of the EC are qualitatively different. Both the LEA and MEA receive strong inputs from the perirhinal and postrhinal cortices. While the LEA receives a stronger input from perirhinal cortex and anterior associational regions such as the medial and orbital frontal cortex, the MEA receives a more robust projection from the postrhinal cortex, and posterior associational regions like retrosplenial cortex (Burwell and Amaral, 1998b; Naber et al., 1997). It must be stressed that layer III neurons of MEA are also heavily influenced by presubicular input (Caballero-Bleda and Witter, 1993), which conveys more visuo-spatial information from retrosplenial and visual cortices, and dorsal thalamic inputs (head-direction cells which code for the allocentric direction of the animal's head have been found in the presubiculum of the rat; Taube et al,1990a,b).

In conclusion, two parallel streams of information can be recognised within the hippocampal formation, one originating from the MEA and most likely involved in coding of spatial orientation, the other from LEA, processing object-context relations. These parallel streams remain segregated at the CA1 and subicular level, while they are integrated at the dentate and CA3 levels.

Afferents to the hippocampal formation

Cortical afferents

The major input to the hippocampal formation comes from the superficial layers of the EC (layers II and III), through the perforant pathway, whose main organising principles have been outlined in the previous section. Much of this entorhinal input to hippocampus comes in turn from postrhinal and perirhinal cortices (Burwell and Amaral, 1998a), and both these rhinal cortices project directly to CA1 and subiculum (Naber et al., 1999, 2001).

Cortical afferents to rhinal cortex

The rhinal cortices receive most of their inputs from unimodal association and polymodal association areas, suggesting that the hippocampus receives abstracted, highly-processed information from neocortex, but there is also some specialisation of inputs associated with each cortical area.

Perirhinal cortex receives a substantial olfactory input from piriform cortex (which accounts for 50% of its unimodal associational input), while the rest of its unimodal associational input is distributed across all remaining sensory modalities (auditory, visual, visuo-spatial and somatosensory). Postrhinal cortex receives strong input from visual and visuo-spatial association cortex, but only weak inputs from auditory regions and the other sensory modalities.

However, the bulk of cortical input to rhinal cortices comes from polymodal associational cortices, in particular from ventral temporal areas (Tev as defined by Swanson). Perirhinal cortex receives from the whole extent of Tev, while post rhinal cortex mainly from its most caudal aspect, which in turn receives visuo-spatial inputs.

The EC also receives direct cortical inputs. Interestingly, the direct associational input to LEA is more similar to that of perirhinal cortex, while that to MEA is more similar to that of postrhinal cortex. This strengthens the idea that there are two parallel streams of information coming from the peri- and postrhinal cortices, carrying distinct, highly-processed information to fields CA1 and subiculum in the hippocampal formation, by way of the medial and lateral components of the perforant pathway.

Presubiculum and parasubiculum: cortical inputs via entorhinal cortex

Another source of cortical inputs to the hippocampal formation, via the EC, is represented by the pre- and parasubiculum (see fig I.4).

The presubiculum receives a strong projection from retrosplenial cortex (Swanson and Cowan, 1977; Van Groen and Wyss, 1990a,1990b), from visual area 18b (Vogt and Miller, 1983) and from cingulate cortex (Van Groen and Wyss, 1990b). Cells in layer V of the restrosplenial cortex project to layers I and III-V of

the presubiculum. Similarly, cells in layer V of area 18 sends projections that terminate in layers I and III, and are more prominent to the dorsal portion of the presubiculum. Cortical inputs to the parasubiculum are similar, but lighter than those to presubiculum and include occipital visual areas and retrosplenial cortex (Van Groen and Wyss, 1990a, Vogt and Miller, 1983).

Presubicular output is primarily directed to layers III and I of entorhinal cortex, and to medial entorhinal cortex only (MEA; Kohler, 1985). So, through the presubicular input, more visual information enters the medial perforant pathway, targeting specifically the proximal half of CA1(next to CA3) and the distal half of the subiculum (next to presubiculum).

Presubicular layers II and III originate the presubicular-entorhinal projections, which have been shown to be mainly excitatory, targeting both principal neurons and interneurons bilaterally in the MEA. It is important to note that 20% of the dorsal presubicular projections to MEA are inhibitory in nature and most likely target inhibitory interneurons in the ispilateral dorsal MEA (van Haeften et al, 1997).

Presubicular layer V cells project to retrosplenial cortex layers I and II, reciprocating the retrosplenial to presubicular connections (Van Groen and Wyss 1990a).

Parasubicular output is directed at layer II of both medial and lateral entorhinal areas. It is important at this stage to point out the existence of a dense projection from parasubiculum to dentate gyrus (Kohler, 1985, Witter et al., 1988).
There is a pattern whereby, through entorhinal cortex, parasubiculum targets early stages of hippocampal processing (DG and CA3), and presubiculum targets later stages (CA1 and subiculum).

Both pre- and parasubiculum receive a substantial input from the subiculum which terminates mainly in layer I of these structures. The subicular projections to the dorsal presubiculum terminate somewhat differently, innervating preferentially layer V and to a lesser extent layers I and II (Kohler, 1985, Swanson et al, 1978; van Groen and Wyss, 1990a,b). There are rather weak reciprocal projections from pre- and parasubiculum back to the subiculum (Kohler, 1985).

Pre- and parasubiculum project to the molecular layer of the dentate gyrus, and the parasubicular projections are much denser than those from the presubiculum (Kohler, 1985; Witter et al, 1988). Thus the parasubiculum might be able to influence hippocampal information processing through a double route, one by way of the entorhinal cortex, the other through the dentate gyrus.

Pre- and parasubiculum also project, albeit rather weakly, to all fields of the hippocampus (Kohler, 1985; Witter et al, 1988).

There are reciprocal connections between pre and parasubiculum. Cells throughout the presubiculum project to layers I and II of the parasubiculum (Kohler, 1985, Van Groen and Wyss, 1990a), while parasubicular projections target layers I and III of the presubiculum (Kohler, 1985, Van Groen and Wyss, 1990a).

Pre- and parasubicular intrahippocampal connectivity suggest that they lie at the crossroad between output and input, given that they receive strongly from the subiculum and project heavily to the entorhinal cortex and, in the case of parasubiculum directly to the dentate gyrus. This functional loop might be important in re-channelling into the hippocampus information already processed by the hippocampus itself.

Subcortical afferents

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

An important thalamic input reaches the hippocampal formation by way of the pre- and parasubiculum. Presubiculum is reciprocally connected to all nuclei of the dorsal thalamus. Ventral presubiculum receives most of its input from the laterodorsal and anteroventral nuclei, whereas the dorsal part receives its inputs from the laterodorsal and anterodorsal nuclei (Van Groen and Wyss, 1990a,b, 1995; Shibata 1993). Projections from the thalamic nuclei terminate in layers I, III and IV of the presubiculum (Van Groen and Wyss, 1995), Presubiculum sends projections to the lateral mammillary nuclei (Donovan and Wyss, 1983; Meibach and Siegel, 1975, Swanson and Cowan, 1977). Head-direction cells have been recorded in both the anterodorsal thalamus (Taube, 1995a) and the lateral mammillary nuclei (Stackman and Taube, 1998; Blair et al, 1998). It seems likely that the head direction signal converges on the presubiculum from both these sources and then reaches the hippocampal formation by way of the presubicular projection to layer III of MEA mentioned previously. This is also supported by the fact that: a) there is no direct presubicular projection to hippocampus proper; b) the anterior thalamus projects primarily to deep layers of entorhinal cortex (Shibata, 1993), and 3) there is no input from anterior thalamus to hippocampus (just to layer I subiculum: Van Groen and Wyss, 1995).

Parasubicular thalamic input is similar to that of presubiculum, and includes strong reciprocal connections to the anterodorsal thalamic nucleus, and afferent

connections from the laterodorsal thalamic nucleus (Van Groen and Wyss, 1990a,b, 1995; Shibata 1993).

Efferents of the hippocampal formation

CA1 efferents

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

Subicular efferents

As has been emphasised in the general description of hippocampal connectivity, the subiculum is one of the major output regions of the hippocampal formation. Prominent projections of the subiculum include those to:
a) portions of medial prefrontal cortex, particularly to the medial and ventral orbitofrontal cortices, and to the prelimbic and infralimbic cortices
b) retrosplenial cortex
c) perirhinal cortex
d) medial anterior olfactory nucleus
e) lateral septum
f) nucleus accumbens, particularly its caudomedial part (ie mainly the shell)
g) ventromedial hypothalamic nucleus
h) medial mammillary nuclei (the lateral mammillary nucleus, important in generating the directional signal in anterior thalamus and presubiculum is only sparsely innervated by the subiculum)
i) medial thalamic nuclei including the nucleus reuniens, and interanteromedial nucleus
(Amaral and Witter, 1995; Groenewegen et al, 1987; Naber and Witter, 1998; Witter et al, 1989, Witter and Groenewegen,1990; Wyss and Van Groen, 1992)

Entorhinal efferents: cortical

Deep entorhinal layers receive input from CA1 and subiculum; accordingly neurons in these entorhinal layers may be very important in acting as the hippocampal conduits to the rest of the cortex. It is not clear, however, whether or not the entorhinal cortex of the rat, like that of the monkey (Van Hoesen, 1982), gives rise to prominent and widespread projections to the unimodal and multimodal association cortex (Amaral and Witter, 1995). Swanson and Kohler (1986) suggested that rat entorhinal cortex gives rise to projections reaching a large domain of the cortical

surface, while others suggested that only a very dorsolateral part of the entorhinal cortex projected to widespread neocortical areas (Sarter and Markowitsch, 1985), and it was possible that this region was in fact perirhinal. A detailed study by Insausti et al (1997) reexamined this issue and confirmed that most of the hippocampal-cortical connections in the rat are mediated by way of entorhinal-perirhinal-cortical connections, while only a very restricted part of the entorhinal cortex sends projections that can reach widespread portions of the neocortex (Insausti et al, 1997)

Entorhinal efferents: subcortical

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

Parasubicular and Presubicular efferents

These regions may not be very important outputs for the hippocampal formation as a whole. The parasubiculum serves primarily as an input to the hippocampal formation. It has virtually no efferents to regions outside the hippocampal formation, except to the reciprocal connections to the anterodorsal thalamic nucleus described earlier (Van Groen and Wyss, 1990a, 1995). On the contrary, the presubiculum has a wide set of efferents. These are predominantly to parahippocampal-hippocampal region, but include projections to the retrosplenial and perirhinal cortices, and to regions of thalamus that project to presubiculum (anterodorsal and laterodorsal thalamus). As mentioned before, an important point is that presubiculum, via the fimbria/fornix, projects ipsilaterally to the lateral mammillary nuclei.

Roles of pre and parasubiculum in the parahippocampal-hippocampal network

The general pattern of connectivity of pre- and parasubiculum suggests that their role is one of input to the hippocampal formation.
In particular, the presubiculum conveys a strong, dorsal thalamic input and visual information from visual occipital areas and retrosplenial cortex, to layer III of entorhinal cortex. It is very likely that the head-direction signal enters the hippocampal formation through this connection, given that there is no direct anterior thalamic input to the hippocampal formation, and that the projection of the anterodorsal thalamic nucleus (AD) to the entorhinal cortex is primarily restricted to its deep layers (Shibata et al, 1993). The presubicular input has a strong visuo-spatial content and it is not surprising that it targets selectively the MEA, which, we have seen, is most likely involved in coding of spatial information.

Recently it has been shown that presubicular axons within layer III of entorhinal cortex not only make synaptic contacts with principle cells and interneurons in layer III (van Haeften et al, 1997), but also target dendrites of layer V pyramidal cells (van Haeften et al, 2000). This opens up the possibility that the presubiculum could also influence the output of the hippocampal formation.

While presubiculum targets layer III entorhinal cortex (and thus CA1 and subiculum), parasubiculum projects to layer II of both MEA and LEA. This, coupled with the fact that parasubiculum sends a very dense projection to dentate gyrus, shows that it targets early stages of hippocampal formation.

The hippocampal formation processing may be more strongly influenced by pre- and parasubiculum than previously thought, as suggested by a recent study estimating neuron numbers in these areas: in the rat, together, the presubiculum and parasubiculum contain the same number of neurons (7×10^5) as the MEA and LEA combined (6.9×10^5), parasubiculum contributing about 30% to this total (Mulders et al, 1997).

To summarise, the role of pre- and parasubiculum within the parahippocampal-hippocampal network may be mainly to channel in thalamic input via the superficial layers of entorhinal cortex, and may also provide a way for hippocampal output to be reprocessed as input (pre- and parasubiculum both receive strong projections from the subiculum).

Chapter II

Physiology of the hippocampal-parahippocampal network

This chapter focuses on the physiological properties of the hippocampal-parahippocampal network. The first part deals with the global physiological patterns of the hippocampal system, focusing on the characteristics, the hypothesised generation mechanisms, the behavioural and functional significance of the global EEG states in the hippocampal-parahippocampal system.

The second part looks at the physiological properties of cell types within the parahippocampal area, concentrating on the pre- and parasubicular cortices which are the main subject of this thesis.

Global EEG patterns

Different population patterns are recognisable throughout the hippocampal formation and the parahippocampal region. They result in spontaneous oscillatory activity at different frequency domains, spanning from slow (7-12 Hz, theta), to fast (40-100 Hz, gamma), and ultrafast (200 HZ, "ripples") waves.
These oscillations are the product of synchronous activity within the hippocampal network, reflecting different functional states of the system, each correlating with a specific set of behaviours.

Theta and the nested gamma oscillations occur, in the rat, during motion, exploratory activity, alert attention and rapid eye movement sleep. In contrast, during immobility, consummatory behaviours, and slow-wave sleep, the hippocampus entrains in what has been traditionally described as Large Irregular Activity (LIA; Vanderwolf, 1969). LIA consists of large irregular, slow waves (slower than theta), which, in the deep layers of entorhinal cortex and in stratum radiatum of CA1, translates into a large amplitude (1-3 mV), aperiodic field potential also called the sharp wave (SPW; Buzsaki, 1983). Sharp waves coincide with a high frequency (200 Hz) oscillatory activity in the stratum pyramidale of CA1 and deep layers of several parahippocampal structures, generally referred to as "ripples" (O'Keefe and Nadel, 1978, p.150-3).

SPW and "ripples"

Intermittent population bursts in the CA3-CA1-subiculum-entorhinal cortex axis are associated with sharp waves (SPW) in the dendritic layers, and ultrafast oscillations ("ripples" at 140-200 Hz) in the somatic layers of these structures.

Within the hippocampus, sharp waves take the shape of large negative spikes, best recorded from stratum radiatum in CA1 (just below the pyramidal layer). They are thought to arise from a burst of activity in CA3, that produces a large depolarisation in the Schaffer collaterals' targets, the dendrites of CA1 neurons and interneurons. The synchronous depolarisation of CA1 neurons and inhibitory interneurons results into a high frequency oscillation, the "ripple", above and within the stratum pyramidale of CA1. Both interneuronal and pyramidal firing is phase locked to the rhythmic ripple oscillation, but while each interneuron generally fires several spikes during one ripple event, pyramidal cells tend to fire a single action potential per ripple (Chrobak and Buzsaki, 1996). The mechanism by which this highly coherent discharge of pyramidal neurons is brought about along the whole extension of the dorsal CA1 region is still unknown. One hypothesis assumes that the coherent discharge results from the reciprocal interconnections between pyramidal cells and interneurons. According to this explanation, the CA3 depolarising output induces a fast oscillatory discharge in the interneuronal population. The chance discharge of just a few pyramidal cells would reset the interneuronal oscillatory activity, thus producing a short-lived coherent discharge (Ylinen et al., 1995). Another hypothesis has been put forward more recently and assumes that the synchronisation of pyramidal cells is achieved through axo-axonic gap junctions. Indeed, ripple oscillations persist in the absence of chemical transmission, while inhibitors of gap junctions were shown to block ripple activity in hippocampal slices (Draghun et al., 1998). Moreover, a recent paper by Schmitz et al. (2001) provides both anatomical (even if indirect, using the dye coupling technique) and electrophysiological evidence of the existence of axo-axonic gap junctions between pyramidal cells in *in vitro* hippocampal preparations. Another piece of evidence, which is consistent with the gap junction hypothesis, is that, during a ripple event, interneurons in both stratum pyramidale and oriens fire 1-2 msec after the pyramidal neurons (Csicsvari et al., 1999; although this timing is also compatible with synaptic transmission).

Ripples occur in the same hemisphere, virtually simultaneously, in CA1, subiculum, and in the deep layers of presubiculum. Ripples are also recorded from the deep layers of entorhinal cortex, but entorhinal ripples occur 5-30 msec after the CA1 ripples. In the entorhinal cortex a sharp wave, coincident with the EC ripple can be recorded as a large negative spike in layer III, which reverses polarity within layer II, suggesting it could reflect a large EPSP on the dendrites of layer V and VI cells.
Cells in the deep layers of all these retrohippocampal areas show increased firing during hippocampal sharp waves (Chrobak and Buszaki, 1994), and single units preferentially fire at the negative peaks of the locally recorded ripple (Chrobak and Buszaki, 1996).

Ripples have also been recently described in humans. They are 80-160 Hz oscillations, highly coherent even across hemispheres (Bragin et al.,1999).
As for the functional role that ripples might have, Buszaki (1989) has suggested a model of memory trace formation in the entorhinal-hippocampal region, involving ripples, which will be briefly discussed at the end of next section.

Theta

The theta rhythm is the largest synchronous signal that can be recorded in the normal EEG of the mammalian brain. It consists of large amplitude (1-2 mV) oscillations in the frequency range of 7-12 Hz, and was originally described in the hippocampus of rabbits after nociceptive or auditory stimulation (Jung and Kornmuller, 1938 cited in Green and Arduini, 1954). Since then, its presence has been largely demonstrated in rats, where it can be recorded from several brain regions, including the hippocampal formation, the superficial layers of presubiculum and entorhinal cortex, septum, hypothalamic and brainstem nuclei.
Behaviourally it correlates with exploratory activity, voluntary movements and the rapid eye movement stage of sleep.

The mechanism(s) of theta generation in the hippocampal-parahippocampal region are a matter of intense debate, and the following, brief description of the major controversies related to this issue, cannot possibly be comprehensive. I will discuss the literature with reference to three areas of research: the role of the septum, intrahippocampal theta network and intrinsic oscillatory properties of cells in the hippocampal formation.

The role of the septum

One of the few widely accepted evidences is that reversible or irreversible lesions of the medial septum/diagonal band of broca (MS/DBB) permanently abolish theta rhythm both in hippocampal and parahippocampal areas.

MS/DBB sends both cholinergic and GABAergic projections to the whole extent of the hippocampal formation. The majority of MS/DBB cells (about 75%) fire rhythmically at theta frequency and continue to do so even after deafferentation (Vinogradova, 1995). This septal population of bursting neurons likely includes both cholinergic and GABAergic neurons. Indeed, Lee et al (1994) showed that selective and total lesions of the septal cholinergic input to the hippocampus did not completely eliminate theta, but "uncovered" a low amplitude theta, most likely maintained by the GABAergic septohippocampal projections.

The evidence that lesions of MS/DBB result in complete loss of theta, has inspired the so called "septal pacemaker" hypothesis of theta generation, which proposes that the septal input to the hippocampal formation is the sole generator of theta rhythm.

It must be said at this stage that in vitro studies have shown that the hippocampal formation has intrinsic oscillatory properties. There is multiple evidence that applications of carbachol (cholinergic agonist) to hippocampal slices, induces membrane potential oscillations (MPOs) at theta frequencies in pyramidal cells in region CA1 (Bland et al, 1990; MacVicar, and Tse, 1989), CA3 (MacVicar and Tse, 1989) and the molecular layer of dentate gyrus (Bland et al, 1988). How this relates to the intact animal is controversial. For instance, it has been shown that CA3 does not produce theta rhythm in the intact animal, after carbachol infusions (Bland et al,

1975). Colom et al. (1991) performed an experiment where they lesioned the septal afferents in urethane-anaesthetised animals and demonstrated that application of carbachol alone to the hippocampus failed to induce theta rhythm (in contrast to what has been just described in the *in vitro* preparations). This was attributed to the fact that *in vivo* the inhibitory circuitry is still intact (and presumably stronger than in *in vitro* preparations). To assess this, they administered both carbachol and bicuculline ($GABA_A$ antagonist) to their *in vivo* preparation, and showed that this produced theta-like oscillations in the hippocampus. It is important to note, however, that the amplitude, shape and frequency of these theta-like oscillations differed significantly from natural theta oscillations.

To return to the septal pacemaker hypothesis, several attempts have been made at modelling the exact mechanism by which the septum would entrain the hippocampal formation in the theta rhythm. The model recently proposed by Vertes and Kocsis (1997) is based on the anatomical evidence that while the septal cholinergic projection terminates on all types of hippocampal cells (Shute and Lewis, 1966; Frotscher and Leranth, 1985; Freund and Antal, 1988), septal GABAergic neurons specifically innervate hippocampal GABAergic interneurons (Freund and Antal, 1988; Gulyas et al., 1991). One crucial assumption of the Vertes model is that both cholinergic and GABAergic septal neurons discharge in synchronous bursts during theta. During each burst of activity:

1) the cholinergic input would have a depolarising effect on pyramidal cells and a pre-synaptically mediated inhibitory effect on GABAergic interneurons in the hippocampus.
2) GABAergic MS/DBB would inhibit hippocampal GABAergic interneurons.

The coordinated action of these two septal inputs would strongly excite pyramidal cells and generate the negative peaks of the extracellularly recorded theta rhythm. During the septal neurons interburst period, pyramidal cells would be inhibited again by the network of hippocampal GABAergic interneurons, and this would explain the positive peaks of theta.

There are two problems with this model. One is that there is no conclusive evidence that MS/DBB neurons fire coherently (phase-locked) to the theta rhythm (Stewart and Fox, 1989; Lee et al. 1994; Brazhnik and Fox, 1997; Gaztelu and Buno, 1982; Stewart and Fox, 1990; King et al., 1998). The other is that the model predicts that hippocampal interneurons and pyramidal cells would fire at different phases of the theta rhythm (on the positive and negative peaks of the theta oscillation respectively). While this has been shown to be true in urethane-anaesthetised rats, there is ample evidence that in the awake animal both pyramidal neurons and interneurons fire preferentially at the negative peaks (troughs) of the extracellularly recorded theta rhythm (Csicsvari, 1999, Fox et al. 1986). Moreover pyramidal neurons show phase-shift in the awake and behaving animal (O'Keefe and Recce, 1993; see chapter IV p. 33), while the firing of hippocampal interneurons is phase-locked. This does not discount the pace maker hypothesis of theta generation, but shows how far we are from a detailed description of the theta phenomenon.

Intra-hippocampal theta network

The idea that there might be intrinsic hippocampal generators of theta results from current density analysis of electrical activity in the hippocampus. In particular a study by Buzsaki et al. (1986) demonstrated a source of current in the pyramidal layer of CA1 accompanied by current sinks in the distal apical dendrites and the outer third of the dentate molecular layer. About half a theta cycle later, a current sink appeared in CA1 stratum oriens, together with a deep sink in the middle third of the dentate molecular layer. This was interpreted as supporting the thesis that hippocampal theta rhythm is the result of two inputs: one from the medial septum, and one from the theta generator in the entorhinal cortex. A later study by Brankack et al. (1993) revealed a prominent source at the level of the pyramidal cell bodies and the hilus, as well as a number of sinks in the stratum radiatum, the apical dendritic tree of CA1 and the stratum moleculare of the dentate gyrus. In this paper it was proposed that, in addition to the excitatory inputs from the entorhinal cortex, the excitatory connections between cells in the 'tri-synaptic loop' (the dentate gyrus to CA3 to CA1 pathway) also provide rhythmic inputs.

Following the idea of intrahippocampal theta generators, in a recent paper Kocsis et al. (1999) have shown that there is a highly coherent coupling between theta signals recorded from: 1) the hippocampal fissure and str. oriens of the CA1 region and 2) between CA1 str. radiatum and the dentate molecular layer. After bilateral, complete lesions of the entorhinal cortex, all the signals recorded from both below and above the CA1 pyramidal layer become highly coherent. The coherence between the signals from str. oriens and the hippocampal fissure was interpreted to reflect the perforant path excitatory effect on the distal dendrites of CA1 pyramidal cells and the coordinated somatic inhibition of CA1 pyramids mediated by the perforant path activation of basket and chandelier cells in stratum lacunosum molecolare, which in turn would inhibit the soma of CA1 pyramidal cells. In this model, one does not need the septal pacemaker to coordinate the somatic inhibition and distal dendritic depolarisation of CA1 cells, because they both result from the entorhinal projection. As for the coherent signals recorded from CA1 str. radiatum and the dentate molecular layer, Kocsis et al., attribute these to the associational-commissural afferents originating from the CA3 pyramidal cells and dentate mossy cells which target, respectively, the CA1 stratum radiatum and the inner molecular layers of the dentate. After bilateral entorhinal lesions, this theta generator is uncovered, and theta signals become highly coherent throughout the CA1 to CA3 to dentate axis. This model might also explain the results of the *in vitro* data described before, implying that in *in vitro* preparations, extrahippocampal theta inputs are not present, and the application of carbachol to the slice activates the CA3-mossy cell theta oscillator.

Intrinsic oscillatory properties of hippocampal cells

Intracellular oscillations were first observed in hippocampal pyramidal cells by Fujita and Sato (1964). They found a rhythmic membrane potential oscillation (MPO) in cells from CA1, CA2 and CA4 that was synchronous with the theta rhythm recorded extracellularly in the pyramidal layer. They showed that these MPOs were the result of EPSPs. It is still controversial if the nature of intracellular MPOs

recorded from cells within the hippocampal formation reflect EPSPs or IPSPs. This issue lies at the heart of attempts to determine the mechanisms underlying hippocampal theta rhythm and it has received experimental attention from many different laboratories. In their exhaustive review Vertes and Kocsis (1997) propose that the controversy is due to the nature of the preparation used (curare-anaesthetised vs urethane or ketamine anaesthetised animals). As we have already described, according to their thesis, in awake animals theta reflects both positive-going (depolarising membrane potential) and negative-going (hyperpolarising membrane potential) oscillations.

The firing of cells throughout the hippocampal-parahippocampal formation is modulated by the theta rhythm. Firing of the vast majority of dentate granule cells (83% Rose et al., 1983) shows a strong relationship to theta, and it has been shown that putative granule cells preferentially fire on the descending (positive-to-negative) phase of the extracellularly recorded CA1 theta wave (Fox et al, 1986, Buzsaki et al, 1983). In the hippocampus proper Ranck (1973) subdivided neurons into two broad classes: 1) pyramidal neurons, which fire at low rates (generally around 2 Hz and not greater than 30 Hz), have long duration action potentials (0.4-1.2 ms), and show complex spikes, bursts of two to ten spikes of decreasing amplitude and an interspike interval between 2 to 6 ms; 2) interneurons, which fire at high rates (10 Hz to > 30 Hz), have short action potentials (<0.35 ms), and fire single spikes in bursts, synchronous with theta. Interneurons double their firing rates in the presence of hippocampal theta rhythm and thus have been termed "theta" cells. In early and recent studies it has been shown that both pyramidal cells and interneurons within the hippocampus proper show theta modulation of their firing and preferentially fire at the negative peak of the extracellularly recorded CA1 theta wave (Fox et al., 1986, Csicsvari, 1999). In particular, interneurons recorded from the stratum oriens and the stratum pyramidale of region CA1 fire, respectively, 60 and 20 degrees before the pyramidal cells during the theta cycle (Csicsvari et al 1999). Hippocampal pyramidal cells show a peculiar relationship between their firing and the concurrent theta oscillation, firing at progressively earlier phases of the theta wave, as the animal crosses the place field (see chapter IV; O'Keefe and Recce, 1993). This phenomenon of "phase-shift" suggests that hippocampal pyramidal neurons (place cells) might encode information using both a rate (rate of firing) and a time (phase of theta oscillation at which firing occurs) code.

A very small proportion of hippocampal interneurons substantially decrease their firing rate during theta periods (Fox and Ranck, 1975, Buzsaki et al 1983, Colom and Bland, 1987). These cells have been termed "antitheta" or "theta-off" cells and it has been suggested that they are inhibited by septohippocampal afferents (Smythe et al, 1991).

Neurons in the superficial layers of retrohippocampal structures (presubiculum, parasubiculum and entorhinal cortex) show theta modulated firing (Chrobak and Buszaki 1994). This is in contrast to the behaviour of neurons located in the deep layers of the same retrohippocampal structures, whose firing strongly increases in concomitance with hippocampal SPWs, as we have described in the previous section.

Buzsaki et al (1989) have proposed a two-stage model of memory formation, which hypothesises that information is initially encoded within hippocampal circuits

during theta activity (a process involving the "input"/superficial layers of hippocampal and retrohippocampal structures); this information is subsequently consolidated within intra- and extrahippocampal circuits during the SPW states, by way of the activation of the "output"/deep layers of the hippocampal-parahippocampal network.

Theta oscillation has been implicated in gating or facilitating synaptic modifications, which underlie long-term potentiation (LTP). It has been shown that theta-pattern stimulation induces LTP in vitro (Rose and Dunwiddie, 1986). This stimulation protocol has been referred to as Primed Burst stimulation and consists of a single priming pulse, followed 140-170 ms later by a high-frequency (100 Hz) burst of two to 10 pulses. It has also been reported that LTP is preferentially induced by burst stimulation on the positive phase of the theta rhythm in urethane-anaesthetised rats (Pavlides et al., 1988). Moreover, delivery of single pulses phase-locked to a positive theta peak induces LTP, while stimulation on the negative phase has no effect or occasionally induces LTD (long term depression, Huerta and Lisman, 1993).

Gamma

Gamma oscillatory activity was first reported, in the hippocampus, in the hilar region of the dentate gyrus (Bragin et al., 1995), and it has been later described in the superficial layers of entorhinal cortex (Chrobak and Buzsaki, 1998). Like theta, gamma oscillations show a high degree of coherence along the longitudinal axis of the hippocampal formation, along distances of up to several mms, but their average coherence decreases rapidly along the CA3-CA1 direction.

Gamma oscillations occur during theta-associated behaviours (exploration, sniffing, REM sleep), and are nested to the concurrent theta waves, their frequencies span from 40-100 Hz and there is a high correlation between theta and gamma frequency shift. This suggests that similar, still obscure, mechanisms modulate the frequency of both gamma and theta rhythms.

Unlike theta, gamma oscillations persist after removal of all subcortical inputs to the hipppocampal formation (Buzsaki et al, 1987), demonstrating that they are generated within the hippocampal circuits. A definite source of gamma rhythm is the entorhinal cortex, but after bilateral removal of this input gamma activity is still present in the hippocampus and it is most likely sustained by the CA3 region (Bragin et al., 1995).

Putative interneurons in the hilar region of the dentate gyrus fire phase-locked to the locally recorded gamma oscillations, firing preferentially at its negative peaks. Neurons in the superficial layers of both presubiculum and entorhinal cortex show a similar pattern of firing preference to the gamma oscillations recorded from layer II of entorhinal cortex. It is important to note that gamma oscillations in the dentate hilar region and entorhinal cortex are coupled and in one case where the recording electrodes were placed in anatomically interconnected entorhinal-dentate regions, were shown to be highly coherent (Chrobak and Buzsaki, 1998).

Brief gamma activity periods are also observed in the freely behaving rat as a tail of activity following SPWs (Traub et al., 1996).

The function of gamma oscillations might be synchronising the activity among different subset of neurons within very short time windows (10-25 ms), complementing the role that theta waves have over longer time scales.

Electrophysiological characteristics of pre- and parasubicular neurons

There are very few studies aimed at delineating the basic electrophysiological and morphological characteristics of neurons in the pre- and parasubicular cortices, and the following description derives from the work of Makoto Funahashi and Mark Stewart (1997a-b, 1998) on horizontal slices of rats' hippocampi. Their main finding is that cells show different electrophysiological properties depending on their laminar origin, rather than their area of origin (presubiculum vs parasubiculum), or their morphological appearance (stellate vs pyramidal cells). In particular, cells within the deep layers of both pre- and parasubiculum, show quite different characteristics from cells in their superficial layers. This is in line with the two-stage theory of memory formation, proposed by Buzsaki (1989) and outlined in an earlier section, which rests on the assumption that there is a strong functional segregation of deep vs superficial layers within retrohippocampal structures.

At the morphological level, cells in pre- and parasubiculum can be classified into two categories: pyramidal and stellate cells. Pyramidal cells are found in layer II and V of parasubiculum and layers III and V of presubiculum. Pyramidal and stellate neurons are generally considered to be projecting and inter-neurons respectively, but, importantly Funahashi and Stewart, in their 1997 paper state:
"It has been reported that stellate-like interneurons in CA1 showed very short duration action potentials and no adaptation of firing response to current injection (Lacaille and Williams, 1990). While we cannot rule out the possibility that some of our stellate cells were interneurons, none had the firing properties just described".

In contrast to cells recorded from the subiculum, CA3, and many neocortical areas, pre- and parasubicular cells respond with single spikes to current injections, never showing bursting responses. Again, deep layer cells differ as a group from superficial cells in that they show greater spike frequency adaptation than superficial layer cells. A bursting response can be elicited, only in the deep layer cells, through extracellular stimulation of the subiculum, deep medial entorhinal cortex or superficial pre- or parasubiculum. This *in vitro* burst response involves *all* the neurons in the deep layers and is thought to be the equivalent of the sharp wave activity observed in the same areas in the awake animal (Chrobak and Buzsaki, 1994, 1996). Very interestingly, this very powerful excitatory activity in the deep layers never propagates to the superficial layers of pre- and parasubiculum. This could be due to the strong inhibitory control that superficial layers of all retrohippocampal structures are thought to be under. Evidence in this direction comes from converging morphological, immunohistochemical and electrophysiological studies. It has been shown, for instance, that in the rat, while neurons and terminals which stain positively for GABA or glutamic acid decarboxylase (GAD) were found throughout all retrohippocampal cell layers, they were more numerous in the superficial cell layers II, III, particularly in entorhinal cortex (Kohler, 1985). This finding has been more

recently replicated in the macaque monkey (Jongen-Relo et al., 1999), where the highest density of GABAergic neurons in the hippocampal formation was found to be in the presubiculum, and in particular in its layer II. Parallel electrophysiological findings show a prevalence of inhibitory responses in the superficial layers of both entorhinal (Jones et al., 1993) and pre- and parasubiculum (Funahashi and Stewart, 1998).

The electrical segregation of deep and superficial neurons within pre- and parasubiculum might reflect, in addition to the powerful inhibitory control just described, a peculiar anatomical organisation of these cortical areas. Funahashi and Stewart (1997b) have suggested that, while superficial layers of pre- and parasubiculum send descending projections to deep layer neurons, the reciprocal, ascending connection (deep to superficial), does not exist. Their claim is based on both morphological (Golgi staining) and electrophysiological evidence (antidromic activation of superficial neurons from deep layers stimulation). This arrangement would be peculiar to pre- and parasubicular cortices, because reciprocal deep-to-superficial connections have been shown within the entorhinal cortex in anatomical studies (Dolorfo and Amaral, 1998b).

In summary, the work of Funahashi and Stewart on the electrophysiological properties of pre- and parasubiculum, strongly supports the idea of a functional segregation between the deep and superficial layers of these retrohippocampal structures, and are consistent with similar electrophysiological evidence gathered from the study of the entorhinal cortex.

Chapter III

Functional studies of pre- and parasubiculum

Only a handful of studies have explored the issue of the specific functional roles of pre- and parasubicular cortices within the hippocampal-parahippocampal network. This is probably partly due to the relative difficulty in selectively lesioning these two structures, while sparing the adjacent medial entorhinal area.

In an early study, Taube et al. (1992) showed that rats with bilateral electrolytic or neurotoxic lesions to the dorsal presubiculum (referred to as post-subiculum) performed poorly on spatial memory tasks, while their performance was normal on non-spatial tests. The tasks chosen were the radial eight-arm maze, and the Morris water maze task, in its reference memory and cued version. In the radial 8-arm maze task the animal is required to visit the 8 arms of the maze (which are all rewarded), without re-entering an already visited arm. It is a spatial working memory task, and it has been shown in the past that generally rats solve the task using an allocentric (world centred) strategy. The observation that rats do not show any specific order in the pattern of arms selected, makes it very unlikely that they are using an egocentric (body centred) strategy (for example, consistently making right or left body turns).

In the reference memory version of the Morris water maze task, the animal has to swim, in a pool of opaque water, to a hidden platform located in a fixed position in the pool. The starting position is changed at every trial, so the animal cannot solve the task by learning a stereotyped set of motor behaviours to reach the platform. To monitor the amount of learning during training, the time the animal takes (latency) to swim to the platform is recorded. At the end of training, the animal's ability to locate the platform is tested in a probe trial where the platform is removed altogether and, to assess the extent of spatial information acquired during training, various parameters are recorded: 1) the time the animal takes to reach the area where the platform was located during training, 2) the time it spends in that area, and 3) the number of times it crosses the region were the platform was located.

There is an extensive literature which shows that this task is sensitive to hippocampal lesions (Morris er al,1982; Morris et al 1990; Riedel et al, 1999). In the cued version of this task the platform is made visible, and its position is made prominent with the use of visual cues attached to it. In this case, all the animal is required to do is to orient to the visual stimulus and reach the platform. Hippocampectomised animals have no problems in solving this task.

In Taube's study (1992), animals with bilateral lesions restricted to the dorsal presubiculum were impaired in the acquisition of both the radial 8-arm maze and the reference version of the Morris water maze task. Although they showed some degree of learning, their performance was significantly lower than that of controls at all time points during training. In the probe trial of the Morris water maze both the number of crossings and the time they spent in the correct quadrant of the pool were significantly

lower when compared to controls. Interestingly, unlike hippocampal animals, post-subicular lesioned animals showed a localised searching strategy during the probe trials, indicating that during training they had acquired some degree of knowledge of the position of the platform. Lesioned animals were unimpaired in the cued version of the Morris water maze task, demonstrating that they did not show any motor or perceptual impairment that could account for their poor performance in the spatial (reference) version of the task. They were also tested in a conditioned food-aversion task and showed no impairment. In all, these results indicate that the dorsal presubiculum is involved in processing spatial information and is required for both reference and working spatial memory.

The involvement of presubiculum in spatial working memory is also suggested by a recent study by Kesner and Giles (1998). In this case, electrolytic lesions encompassed both pre- and parasubiculum bilaterally, and the rats were tested on a continuous recognition memory task in the radial 12-arm maze. This task is similar to the radial 8-arm maze task, in that the animal has to remember which arms he has already visited and avoid re-entering them. The difference is that in the 12-arm maze task, doors prevent the access to the arms, and only one door at a time is opened. In the course of each session, 12 arms are sequentially presented (the door is opened), and of these 12 arms, only 3 to 4 are repeated (and consequently not rewarded). The repetitions are presented with variable lags, from 0 to 6, where a lag of 0 indicates that the arm is repeated immediately after the first presentation and a lag of 6 indicates that there are six different arm presentations between the first and the repeated presentation. This allows one to monitor the performance of the spatial working memory system at several delays. Rats with pre- and parasubicular lesions showed a profound impairment in this task at all time lags. Interestingly animals with lesions restricted to either the medial, or the lateral entorhinal cortex showed no impairment whatsoever. The control task was a go/no go task, in which rats were trained to enter only one of two fixed arms (presented in a pseudo-random order). Animals with lesions in both pre- and parasubiculum could solve this task. This controls for perceptual impairments and excludes also the possibility that pre- and parasubicular lesioned animals fail to acquire the 12-arm maze test because of a deficit in response inhibition.

The most extensive behavioural study of pre- and parasubicular lesioned rats has been conducted by Liu et al. (2001). The animals sustained selective and bilateral neurotoxic lesions (ibotenic acid) to the pre- and parasubiculum, and were tested on a battery of spatial and recognition memory tests which ranged from the Morris water maze task (in its reference, cued and working memory versions), T-maze alternation task, and object recognition memory tests.

The results of the Morris water maze test mirror the ones already described in the study by Taube (1992), with an interesting addition. The animals were given two probe trials, one following the reference memory training, the other following the cued version of the task. Intriguingly, the lesioned animals were selectively impaired only on the reference probe trials, and performed at the same levels as the controls on the probe trial which followed the training in the cued version. This implies that during the cued presentations, with the platform in full view, the lesioned rats learned something about the platform location that they failed to learn during the hidden-platform training. This is similar to what happens in animals with hippocampal

lesions, although they seem to require longer exposure to the visible platform than the pre-/parasubicular lesioned animals, see Whishaw and Jarrard, 1996.

The animals were also tested in a working memory version of the water maze. In the working memory version of the Morris water maze tasks each trial consists of two phases, a sample and a test phase. Between each trial the position of the hidden platform is changed, and the animal starting position is different for each phase. The animal is required to find the hidden platform during the sample phase of the trial, and then re-locate it, starting from a different position in the pool, during the test phase. Between the sample and the test phases a delay is introduced, to test spatial working memory at several time delays (the ones used in this study were 30 and 180 seconds). Pre-/parasubicular lesioned animals were impaired at both the 30 and 180 seconds delay, confirming the role of these structures in spatial working memory. The rats were also tested on the T-maze alternation task, which is considered to be the most sensitive task to hippocampal deficit. It consists of a sample and a test phase; during the sample phase the animal is placed on the start arm (the stem of the T maze) and only one of the two arms is accessible to him and is rewarded. On the test phase the animal is placed again on the start arm, but this time both arms are made accessible, but only the non-rewarded arm during the sample phase is now rewarded. This is also referred to as a Delayed Non Match to Place (DNMP) task, in which the animal has to learn to alternate between the two arms. A delay can be introduced between the sample and test phase and the authors chose to use 5, 30, 90 and 180 seconds delays. Lesioned animals took longer to learn the task (they needed significantly more trials than controls to reach criterion) and were impaired at all but the 5 seconds delay on the working memory version of the task. The authors also took pains to show that both groups of animals were using extra-maze cues to solve the task (and not just an egocentric strategy). The task involved rotating the T-maze in between the sample and test phases. Both controls and lesioned rats were significantly impaired when the T-maze was rotated by 180 degrees, but only lesion animals showed a very mild impairment at 0 or 360 degrees rotations (and this was probably due to the fact that the time delay between sample and test phase was 10 secs, to allow enough time for the maze rotation).

An important contribution of the study by Liu et al. was to test animals on an object recognition task, where animals were introduced in an environment containing 4 objects, and on subsequent exposures the following manipulations were introduced: 1) the location of two of the 4 objects was reversed, 2) a novel object replaced a "familiar" one, 3) one of the objects was either moved towards or away from the other three objects. In all these conditions the time the animals spent exploring the objects was recorded. It should be noted that pre-/parasubicular lesioned animals showed less exploratory behaviour (measured in terms of general locomotor activity and number of rearings) in a novel environment than controls. They also failed to show increased exploratory activity in the displaced, novel and dislocated object conditions described above. These findings were interpreted to indicate that pre- and parasubicular cortices are involved in object recognition memory processes, but they could just reflect a non-selective reduction in exploratory drive (or simply locomotor activity) in the lesioned animals.

In summary, in all three studies a selective deficit in spatial reference and working memory was found in animals with lesions to the pre- and parasubicular

cortices. In particular the deficits in the Morris water maze task are comparable to those found in hippocampal animals, suggesting that pre- and parasubiculum make a strong contribution to water maze performance. In contrast, the deficits shown by pre/parasubicular lesioned animals on the T-maze alternation task are milder than those induced by hippocampal lesion, confirming again the high specificity of this task in testing for hippocampal functioning. The lesion study by Liu et al. might also suggest a role in object recognition memory for pre- and parasubiculum. No attempts have been made so far at disentangling the relative contribution of pre- and parasubiculum to these spatial tasks, and in light of the specific connectivity of these structures to the entorhinal cortex, it would be a very interesting area of research.

In this context it is also worth mentioning a study by Vann et al. (2000), which used the level of expression of c-fos protein (an immediate early gene) as an index of the relative activity of several cortical regions (including pre- and parasubiculum) in response to different spatial memory tests. This study shows that both pre- and parasubiculum were significantly activated (along with regions in the rostral thalamic area and several subcortical areas) when rats were exposed to a radial 8-arm maze test in both a familiar and novel environment. This gives further support to the idea that pre- and parasubiculum contribute to spatial memory.

Chapter IV

Physiological correlates: review of place cells and head direction cells

This chapter offers a review of the firing properties of two classes of cells isolated in the hippocampal formation: place cells and head-direction cells.

Place cells are hippocampal pyramidal cells that fire at relatively high rates (2-20Hz) in a restricted portion of the environment (thus referred to as their place field; O'Keefe and Dostorvsky, 1971; Muller et al, 1987). Place cells have been shown to be the complex spike cells described by Ranck (see chapter III), corresponding to the pyramidal cells in the CA3 and CA1 fields of the hippocampus proper. Cells that discharge in response to the animal's location in the environment have also been described in several other areas of the hippocampal formation: dentate gyrus (Jung and McNaughton, 1993), subiculum (Barnes et al., 1990; Mizumori et al., 1992; Sharp and Green, 1994), parasubiculum (Taube, 1995c), presubiculum (Sharp, 1996), medial and lateral entorhinal cortex (Quirk et al., 1992; Frank et al, 2000, 2001).

Head direction cells fire maximally when the animal points its head in a given compass direction, signalling the allocentric direction of the head of the animal along the horizontal axis. They were first isolated from the presubiculum (Ranck, 1984, Taube et al, 1990a,b). Since then, head direction cells have been isolated from several limbic and extra-limbic areas such as: the anterior dorsal nucleus of the thalamus (AD; Blair and Sharp; 1995, Taube 1995a), lateral dorsal thalamus (LDN, Mizumori and Williams, 1993), the lateral mammillary nuclei (LMN, Blair et al, 1998; Stackman and Taube, 1998), the nucleus of Gudden (Sharp et al, 2001b), the parietal and posterior cingulate cortices (Chen et al, 1994a,b; Cho and Sharp, 2001) and the caudate nucleus (Wiener, 1993).

A brief overview of the principal characteristics of these two classes of cells will be given here together with some considerations regarding the emergence of the positional and directional signals in the rodent brain. This is to provide the appropriate framework within which it will be possible to discuss the relationships between these two spatial systems, which seem to converge in the topodirectional cells, the main topic of this thesis.

Hippocampal place cells

Basic place field characteristics

A place field is the area of any given environment within which a place cell fires maximally. The typical place field is continuous and compact, showing a single

peak that falls off smoothly in all directions, approaching a 2-dimensional gaussian distribution (Muller et al 1987; O'Keefe and Burgess, 1996). Peak firing rates reported are very variable, ranging from 2-20 Hz. This is due to the intrinsic properties of place cells (some fire at higher rates than others) but also to the varied choice of methods and parameters used to display place fields (smoothing, bin size etc.). Outside the place field, place cells show a dramatic drop of firing rate that can approach 0 Hz in well isolated cells.

Not all place fields can be approximated to a two dimensional Gaussian, for instance, it is well established that the shape of place fields is influenced by the shape of the enclosure the animal is in. It is common to observe crescent shaped fields at the edge of cylindrical environments as well as elongated fields (linear) that "hug" the walls of rectangular or square walled enclosures (Muller et al., 1987; O'Keefe and Burgess, 1996).

A single place cell may fire in more than one region of an environment. This phenomenon was initially thought of as an artefact of the recording technique. It is now accepted that a single complex spike cell may show multiple subfields within an environment, even if the quantitative extent of multiple peaks may be not agreed upon (Muller et al., 1987; O'Keefe and Burgess, 1996).

Directionality of place fields

Place fields are usually omni-directional, meaning that any given place cell will fire every time the animal enters the place field, regardless of the direction of travel of the animal or its head orientation. However, when the animal traverses repeated paths, in a stereotyped manner, place fields become directional. The stereotypy in behaviour can be achieved through environmental restrictions or reward-shaping (or both).

On a linear track, where the rat repeatedly runs back and forth between the rewarded ends of the track, the majority of place cells fire only when the animal runs either on the outward or the inward journey, showing marked directionality (O'Keefe and Recce, 1993; Gothard et al., 1996, Huxter et al, 2002). The same is true on the radial arm maze (McNaughton et al, 1983).
Markus (1995) explored this issue in two ways:

1) comparing the directionality of place fields across two differently shaped environments: a cylindrical open field where place fields were omnidirectional, and a plus maze, where the rat trajectory is constrained by the shape of the environment and place fields were very directional;
2) comparing the directionality of place fields across two behavioural paradigms *in the same environment* (cylindrical open field). He first recorded cells while the rats were sampling the environment uniformly, chasing randomly scattered food. He then recorded place cells when the rats where trained to run stereotypically between four locations in the cylinder, where the food was sequentially placed. The proportion of directional fields grew from less than 20% to nearly 40%.

This study suggests that the directionality seen in place fields is primarily attributable to the rat's restricted behaviour and, only secondarily, to environmental constraints.

Place fields and landmarks

It has been repeatedly shown that place fields' angular location may be controlled by distant landmarks. In such circumstances, when the distal cues are rotated by a given amount, place fields can be observed to rotate both in unison and by a similar amount to the controlled cues. Furthermore, simultaneously recorded place cells rotate in unison (Jeffery et al, 1997; Jeffery and O'Keefe, 1999; Muller et al., 1987; O'Keefe and Conway, 1978; O'Keefe and Speakman, 1987). This is a well established finding, and it is true of all the situations where the cues are not in conflict with the other sensory systems (vestibular, motor feed-back, olfactory etc.).

It is interesting to note that the cues must be placed distally in the environment the animal is moving in, in order for them to exert control over the place fields. This has been shown by two studies (Cressant et al 1997; 1999). The authors placed sets of objects (a wooden cone, plastic cylinder, and red wine bottle) in an open cylinder and found that, when centrally placed, the objects failed to control the angular location of place fields, while the same objects placed at the periphery of the cylinder exerted strong stimulus control over angular location.

Place fields stay unchanged when the experimentally controlled visual cues are removed (Muller and Kubie, 1987; O'Keefe and Speakman, 1987). Even in darkness (absolute lack of visual inputs), place cells maintain compact fields. Quirk et al (1990) found that when rats are placed in the open cylinder in the light and then the lights are turned off, place fields stay unchanged and only rarely show angular rotations.

Place cells are multimodal in nature

The experiments just described suggest that place fields, which can be controlled by visual cues, are generally unperturbed by the absence of visual input.
The general rule seems to be that place cells are very opportunistic with regard to sensory inputs (O'Keefe and Dostorvsky, 1971; O'Keefe, 1976; O'Keefe and Conway, 1978).

Some experiments were specifically designed to understand which sensory modalities are involved in establishing and maintaining place fields (Hill et al, 1978; Hill and Best, 1981; Save et al, 1998). Hill and Best (1981) deprived animals of both vision and hearing: place cells were found in these animals. More recently Save et al (1998) compared place cell firing in rats which were surgically blinded one week after birth with that of normal rats. The animals were made to run in a cylindrical enclosure and four objects were placed inside the cylinder. Place cells in control and blind rats were very similar, the only significant differences between the groups being that the fields of blind rats were slightly more directional, and that blind rats' place cells had lower firing rates. Unlike place cells in control animals, place cells in blind rats took longer to start firing at the beginning of any trial, and only did so when the rat touched one of the four objects. This interesting observation suggests that the animals idiothetic system had to be initialised by a tactile stimulus for the cells to start firing.

Place fields spatial distribution

Initially, it was thought that place field characteristics and distribution were homogeneous throughout an environment (O'Keefe, 1976; O'Keefe and Speakman, 1987). Some recent studies have begun to suggest there may be inhomogeneities. Place fields may tend to be preferentially located near the edges of environments (Hetherington and Shapiro, 1997), and above-average numbers of fields may be clustered proximally to salient cues (Hetherington and Shapiro, 1997).

A very important, and controversial question is if there is an anatomical-topographic relationship between place cells and place fields in the hippocampus. The vast majority of studies have not found any such relationship (Muller and Kubie; 1987, O'Keefe and Nadel, 1978; O'Keefe et al, 1998).

Two studies have shown such a relationship exists (Eichembaum et al, 1989; Hampson et al, 1999). It would be important to replicate these studies (given the importance of the issue they raise).

Emergence of place fields in novel environments

There is no general consensus about how place fields emerge in novel environments.

Hill (1978) reported that initial fields (the first time an animal passes through the field) are indistinguishable from fields recorded later on, when the animal is familiar with the environment. This is in contrast with a study by Wilson and McNaughton (1993), which showed that place fields took 6 to 10 minutes to be established, and that these initial fields were not as stable as the ones recorded in a very similar environment that the rat was already familiar with. In their experiment they used a rectangular box with a partition dividing the box into equal halves of about 60 cm in all 3 dimensions. One half of the box was blocked off by a partition (area B). 3 rats were trained in area A of the box for 10 days. Area B was considered to be the "novel" environment.

The results of a study conducted in our laboratory (Lever et al, in progress) represent a mixture of these two opposing studies, but are closer to those of Hill. Rats are introduced *for the first time* in a square enclosure centred within a curtained environment. Rats do not have prior experience of the curtained environment at all. Many place fields emerge within the first 2 minutes of recording (the shortest time possible to get enough spatial sampling) and a high proportion of them are stable across several subsequent exposures to the same environment.

Place fields change with experience

There is increasing evidence of experience-dependent change in hippocampal cells that might suggest a learning process (Bostock et al, 1991; Mehta et al, 1997; 2000; Lever et al, 2002). The studies by Mehta et al. show that while rats were running along linear tracks, place fields developed asymmetries, and increased in size, as running progressed. An NMDA-function antagonist blocked these experience-

dependent changes (Mehta et al, 2000). It is also important to note that this phenomenon does not seem to apply to place fields in open environments and that the effect only lasts for a day. This makes it very difficult to suggest a role for such changes in place fields, in terms of behaviour.

In the Lever et al. (2002) study, rats were exposed to a two geometrically different environments (cylindrical and square walled enclosures) and place cell representations were shown to diverge gradually and incrementally. The divergence occurred in a totally incidental manner, in the absence of explicit reward. The changes were long-lasting, persisting for at least one month, in the absence of exposure to the environments during the delay period. Moreover the "cylinder" and "square" representations were shown to generalise across two environments of similar shape but different material, implying that the hippocampus had encoded the geometric features of the environments it was exposed to.

Anticipatory firing of place cells

A new line of enquiry into the locational coding of place cells was opened up by Muller and Kubie (1989), introducing the timeshift analysis. In this kind of analysis, the time series describing the occurrence of spikes is shifted relative to the time series describing the location (or other observed variable) of the rat. The idea is then to observe the timeshift which most optimises certain measures of spatial coding, such as, for place cells, spatial coherence, field size, information content and so on. When applied to different datasets, this analysis has given divergent results. Muller and Kubie (1989) found that spikes must precede the rat's position by about 120 msec (with a quite large variance) to optimise 3 measures of place field specificity (place field area, patchiness and coherence), while Sharp (1999a) found that spikes should precede the rat's position, at average running speeds, by about 30-40 msec to optimise 4 measures (3 of which were very similar to Muller and Kubie's). These discrepancies might be due to the uncontrolled effect of running speed on the timeshift analysis, as suggested by Sharp (1999a). Sharp (1999a) showed that there is a (small, though significant) negative correlation between running speed and optimal time shift.

The more fruitful results of this line of research come from the application of the timeshift analysis to different areas of the hippocampal formation where spatially localized signal can be recorded from. This analysis has interesting implications in the study of the functional relationship between the different subsets of spatially tuned cells across the hippocampal formation. It has been shown, somewhat surprisingly, that subicular cells anticipate location more than hippocampal cells (Sharp et al 1999a), that CA1 cells anticipate location more than CA3 cells (Muller and Kubie, 1989) and that parasubicular cells lag behind CA1 cells (Taube et al, 1995c). It is hard to reconcile the clear anatomical evidence that the CA3 to CA1 projection is unidirectional, with the findings of the time shift analysis presented above.

The phase shift phenomenon

O'Keefe and Recce (1993) observed an interesting relationship between the theta wave of the EEG and place field firing, in rats running on a linear track. They found that as the rat traversed the place field of any given place cell, the cell fired at progressively earlier phases of the local EEG theta oscillation. They were recording

from CA1 pyramidal cells. Since then the phase shift phenomenon has been observed in DG/CA3 cells, although the phenomenon is less pronounced (the precession occurs over more resctricted phase ranges per any given run within the field; Skaggs et al, 1996; Huxter et al, 2002). O'Keefe and Recce (1993) proposed that the phase precession observed in CA1 was generated by two oscillators of slightly different periods converging onto the hippocampus. One of these oscillators was the theta wave, the other has still to be identified. They also proposed a functional role for phase precession, postulating that it could represent a temporal code which improves the ability of the hippocampus to represent space. Given that place fields on a linear track can be modeled in terms of symmetric gaussians, firing rates do not distinguish between various portions of the edges of a field. Thus phase coding could in principle disambiguate the entry and exit portions of a place field. Jensen and Lisman (2000) showed that the position reconstruction accuracy of an ensemble of place cells recorded on a linear track can be improved by more than 43% using phase data and rate data as compared to using rate data alone. These findings strengthen the hypothesis that phase contributes to hippocampal spatial representation. Another functional role proposed for the phase shift is that it could facilitate the association of temporally sequential events, through an LTP mediated process (Skaggs et al, 1996).

It is not clear whether phase precession occurs when the animal traverses an open field (bidimensional environment; Burgess and O'Keefe, 1996; Skaggs et al, 1996). It appears that phase shift is less robust in the open field, but can be seen in some cells.

Differences between CA1 and CA3 place cells

There are conflicting reports regarding the differences (if any) between place cells in the CA1 and CA3 fields of the hippocampus.

Olton et al (1978) and Muller et al (1987) found that place fields of CA3 cells were indistinguishable from those in CA1. They did not differ with regards to: average firing field area (as a proportion of the environment), average maximum firing rate or in field shape. Some authors have shown that CA3 fields have higher spatial specificity than CA1 cells (Barnes et al, 1990; Markus et al, 1995). In a more recent study, Mizumori et al (1999) have confirmed that CA3/hilar fields have higher information content than CA1 cells (in this study the authors did not attempt to distinguish between pyramidal CA3 cells and what they called hilar cells). They also showed that CA3 fields are more reliable (higher spatial covariance score across similar trials) than CA1 fields. They also showed, interestingly, that a higher proportion of CA3 fields changed during dark trials when compared to CA1 place fields. Moreover, while the CA1 fields that changed in darkness did so by shifting their position, 65% of the hilar/CA3 fields that changed in darkness did so by stopping firing altogether or by starting firing, while being silent in the light condition. This interesting difference must be investigated further given also the results of the most recent lesion and molecular studies involving the relationship between place cells in CA3 and CA1 (discussed later in this chapter).

Place cells from the dorsal and ventral hippocampus

The septal (anterodorsal) and temporal (lateroventral) poles of the hippocampus differ in connectivity and neurochemistry (see chapter I). Briefly, the septal pole receives more sensory input from the external environment than the temporal pole, while the temporal pole receives more input from the rat's internal environment than the septal pole.

Jung et al (1994) recorded from dorsal and ventral CA1 cells, and found significant differences in several measures of spatial coding. Dorsal CA1 cells had more spatial information content per spike, higher spatial information rates, more sparsity, and smaller place fields than ventral CA1 cells. The difference in place field size was marked. On average the place fields of ventral cells were more than 4 times larger in area than those of dorsal cells. Moreover, of cells recorded during slow-wave sleep, 45% in the dorsal CA1 had place fields, while only 18% in ventral CA1 had place fields. The robustness of these results is shown by the fact that all the dorsal/ventral differences persist, even when only those cells simultaneously recorded from the two regions in three rats are included in the dataset (ie. 42 dorsal, and 61 ventral cells). It must be pointed out, however, that Poucet et al (1994) reported no differences in the size and shape of fields from dorsal and ventral sites.

Relationship between place cell firing and behaviour

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

One important finding was that of Markus et al (1994). These authors found that it was the reliability of place cells (ie. their field-stability over time) rather than the spatial specificity of fields that correlated with successful performance on the standard, win-shift, radial maze task (8 arms).

In a more recent study Lenck-Santini et al (2001) recorded place cells while the animals solved a continuous alternation task in a Y-maze with just one prominent visual cue (white cue card). Several sessions were run where the angular position of the cue card was changed or the card was removed altogether. This was done so that on occasional trials the place fields were out of register relative to their standard position. In those instances the rats' performance was significantly decreased, suggesting that there is a strong relationship between place cell representations and

spatial behaviour. The authors also comment on a rather intriguing effect. Rats' performance on the "out-of–register" sessions, though initially poor, improved over time, while place cell firing stayed constant. This demonstrates a dissociation between place cell firing and task performance. However, this very rapid learning might indicate that animals are using the "new" hippocampal representation to solve the task in a very flexible way. Speculatively, the goal position might be being rapidly incorporated into the map.

Jeffery et al (2003) took a different approach with different results. They trained rats to go to a particular corner of a square-walled box (box A) after the occurrence of a tone. This task was shown to be hippocampal dependent. They recorded place cells from these rats in box A. When the animals were introduced to the same shaped box with different walls (box B), the animals' place cells remapped. What was the animals' performance like? The animals' corner choice performance in Box B was significantly impaired relative to their performance in Box A, but was much better than chance (about 63%, chance being 25%). These results appear to show some dissociation between place cell firing and behaviour.

In summary, current indications suggest some caution in attributing causal relationships between place cell firing and overt behaviour.

Location-specific firing in extrahippocampal regions

Dentate gyrus

To date there has been just one study that explored in some depth the physiological correlates of granule cells in the dentate gyrus (Jung and McNaughton, 1993). This is due to the technical difficulties in identifying granule cells. They tend to discharge at relatively low firing rates (the mean rates reported in this study are below 0.5 Hz). The authors solved this problem by defining granule cells on the basis of their evoked discharges to the population spike elicited by perforant path stimulation.

Granule cells show a very moderate theta modulation in their autocorrelograms (lower than that of CA1 place cells) and sometimes fire in Complex Spikes (CS) bursts. As to their spatial selectivity, they show stable place fields on the 8-arm maze and the fields appear to be as directional as CA1 fields under similar conditions. Granule cells place fields are somewhat smaller than those of CA1 cells and a higher proportion of granule cells show multiple sub-fields.

Medial and lateral entorhinal cortex

The entorhinal cortex represents the major input structure to the hippocampus proper, therefore the electrophysiological studies conducted on this area are of crucial importance for understanding where and how spatially selective signals emerge in the hippocampus.

Quirk et al (1992) have recorded cells in the superficial layers of the medial entorhinal cortex that project to the dorsal region of the hippocampus proper. They found that entorhinal units fired in a spatially restricted and reliable way, even though their place fields were bigger and less reliable than CA1 fields. Like CA1 place fields, entorhinal fields followed the rotation of a cue card and kept firing when the card was removed.

The most intriguing difference between entorhinal and CA1 cells was their behaviour when recorded in differently shaped environments. While CA1 fields recorded in a cylindrical environment "remapped" when subsequently recorded in a squared walled environment (provided the rats had been trained for quite a long time in one of these two shapes prior to recording), entorhinal fields "stretched", showing topologically equivalent firing patterns in the cylinder and the square. More importantly, unlike CA1 place cells, some of which stopped firing in this paradigm, no entorhinal cell was ever silent in any of the environments tested.

Two more recent studies have explored the firing properties of neurons in both deep and superficial layers of lateral entorhinal cortex (Frank et al 2000; 2001). In these studies CA1 cells and lateral entorhinal cells were recorded in the same animals, while they ran on W or U (linear) shaped tracks. The results are consistent with those of Quirk et al (1992), confirming that superficial layer entorhinal neurons have lower spatial selectivity than CA1 place cells. Adding to that, the authors showed that the spatial selectivity of deep entorhinal neurons is somewhat higher than that of superficial layer neurons, but still significantly lower than that of CA1 pyramidal cells. On the basis of a theta modulation index measure, both superficial and deep entorhinal neurons were reported to be strongly modulated by theta, even if no such modulation was evident from their autocorrelograms. In agreement with Quirk at al, the authors also suggested that deep entorhinal neurons (and to a lesser extent superficial entorhinal neurons) "represent similarities between locations on spatially distinct trajectories through the environment" (Frank et al, 2000).

Subiculum

The subiculum is the main output structure of the hippocampus. Like entorhinal neurons, subicular neurons have significantly bigger place fields than those in CA1 (Sharp and Green, 1994). In this careful and extensive study no differences in spatial measures (firing rate and spatial coherence) was found between the physiologically distinct categories of bursters, non-bursters and depolarised bursters in the subiculum. One intriguing difference in the spatial responses of the cells recorded, was found along the proximo-distal axis of the subiculum. Spatial coherence, field size, and overall rate measures were higher for cells recorded in the distal as compared to the proximal portion of this structure. This is particularly interesting because it reflects the subicular selective anatomical connectivity referred to in a previous chapter (chapter I). The distal part of the subiculum receives fibres originating in layer III of the medial entorhinal cortex (MEA), which convey visual and visuo-spatial information, while the proximal end of the subiculum is innervated by fibres originating in the lateral entorhinal cortex (LEA), which channel more olfactory and auditory information.

Unlike entorhinal and hippocampal cells, subicular cells do show a weak directional signal, even when recorded in an open environment (cylinder).
Several studies (Sharp and Green, 1994; Sharp, 1997; Sharp, 1999b) have been conducted to explore the nature of subicular spatial representations of differently shaped environments. They indicate quite convincingly that subicular cells, like entorhinal and unlike hippocampal cells, tend to generalise across environments, stretching a "universal" map into the geometrically different environments tested.
In particular, subicular cells showed similar firing patterns in cylindrical and rectangular environments (Sharp and Green, 1994), and in cylindrical and square enclosures that were both geometrically and visually distinct. Hippocampal CA1 cells showed distinct firing patterns when tested under similar conditions (Sharp, 1997). It is crucial to note here that in all these experiments (Sharp, 1997; Sharp, 1999b; Quirk et al, 1992) the authors did not make an explicit attempt at controlling for the amount of experience the animals received before the subicular/entorhinal/CA1 cells were recorded. This is of crucial importance if one considers the results by Lever et al (2002), which show that the CA1 representations of a square and cylindrical enclosures diverge over time, in an experience-dependent manner.

It would be very interesting to conduct similar experiments while recording from entorhinal and subicular neurons, to establish if and after how much experience the entorhinal and subicular representations would differentiate between these two environments. This kind of experiment would shed light on the functional relationships between these three subsystems of the hippocampal formation.

Pre- and parasubiculum

The pre- and parasubiculum lie anatomically at the interface between the input and the output regions to the hippocampus proper (see chapter I, p.14). They both receive strong projections from the subiculum (the major output structure of the hippocampus), while at the same time projecting heavily to the entorhinal cortex (which is the main input structure to the hippocampus). Given the pivotal position they occupy in this structure, it is surprising how little attention they seem to have received.

There are plenty of studies that explore the characteristics of head direction cells first isolated in this area by Ranck (1984).

The study by Sharp (1996) is particularly relevant to this section, because it surveyed the spatial responses of 71 presubicular cells while the rats foraged in a cylindrical enclosure. Sharp applied a multiple regression analysis to this cell population, on the basis of which she divided the cells into 6 categories depending which of the spatial variable considered accounted for the highest proportion of the cell's firing variance. 37% of the cells were classified as "classic" head direction cells, of the type already described in the presubiculum. 17% fell into the "head direction cells with multiple peaks" category. Even though these cells showed as their primary correlate head direction, they were tonically active and unlike head direction cells presented several peaks of high firing at different head angular positions. Other correlates were place (10%), angular velocity (10%) and running speed (7%, one of which, curiously, showed a negative correlation with running speed). One category of cells that deserves further mention is the "place by head direction". This category

includes the highest number of cells (excluding the head direction class), representing 20% of the cells recorded. These cells' firing was described to be modulated by both the animals' head direction and location. They also showed a very weak modulation by angular velocity and running speed. While these cells show similar spatial correlates to the topodirectional cells discussed in this thesis, it must be noted that only 2 out of the 14 cells included in this category by Sharp showed theta modulation. In contrast, theta modulation is one of the hallmarks of topodirectional cells' firing, suggesting that Sharp was sampling from a different cellular population from the one presented here. Another consideration relates to the nature of the regression analysis applied in the Sharp study. While this type of approach seems completely appropriate for classification purposes, it is inadequate in trying to tease out the relative influences of the spatial variables examined over the cells firing rate. The multiple regression analysis suffers also from being influenced by the size of the time bin used, as noted by Sharp (1996).

Taube (1995c) recorded from the parasubiculum in freely-behaving rats foraging in a cylindrical enclosure. He classified 10% of the 174 cells recorded as place cells. Unlike hippocampal place cells, parasubicular place cells never fired in CS bursts. They had a higher background firing rate and larger place than hippocampal place cells fields (covering 47.7% of the environment, against the 13.8% of CA1 place cells). Only 3 out of the 18 place cells recorded were judged to have a secondary head direction component. 41.4% of the recorded cells in parasubiculum were classified as theta cells, a proportion much higher than the one reported for the presubiculum (15%, Taube et al, 1990a; 17% Sharp, 1996) and the subiculum (11%, Sharp and Green, 1994). Taube (1995c) performed a timeshift analysis of the type described before, and showed that parasubicular place cells anticipate the rats' position of just 40 msec, "lagging" behind hippocampal place cells. This result suggests that the parasubicular place cells receive their locational signal from the hippocampus, and not vice versa. In this context it seems counterintuitive that subicular place cells have been reported to anticipate the animal's future location by a larger amount than CA1 place cells.

Locational signals in the hippocampal formation, functional considerations.

To date only few attempts have been made at trying to define the different roles of the various structures within the hippocampal formation where locationally selective firing has been described. We have a patchy knowledge of the interplay between these structures and how the place signal is generated in the hippocampal system.

Mc Naughton et al (1989) injected colchicine in the dentate gyrus, causing an almost complete loss of dentate granule cells. These lesions severely disrupted spatial behaviour (assessed through the circular platform, the Morris water maze and the radial arm maze tests), while leaving most of the spatial selectivity of CA3 and CA1 place cells unchanged. One of the few, rather intriguing differences they found was that in lesion animals place cells tended to show a stronger rhythmicity in the theta range (7 Hz) as compared to the controls.

More recently two studies have addressed the role CA3 plays in the generation of the place signal in the CA1 field of the hippocampus (Brun et al, 2002; Nakazawa et al., 2002). Nakazawa et al (2002) produced a mutant mouse which selectively lacked the NR1 subunit of the NMDA receptor in the CA3 sub-field of the hippocampus. They recorded from CA1 pyramidal cells and interneurons while the mice where foraging in a cylindrical environment, placed within a curtained enclosure which contained four prominent visual cues. No difference was found in mean firing rate, spike width and place field size between control and knock-out (KO) place cells, while there was a significant decrease in the occurrence of complex spikes for the place cells and in firing rates of the interneurons of KO animals as compared to controls. The authors suggested that the decrease in burst activity of CA1 pyramidal cells and in interneuronal firing rate could be due to the decreased excitatory drive of these cells from CA3.The main finding of Nakazawa et al (2002) is that when place cells were recorded in trials where three of the four visual cues were removed, the firing rate of CA1 place cells in KO animals was dramatically reduced, even though the spatial selectivity of the cells remained intact. Place fields did not shift position, but had a much lower firing rate in the single-cue condition than in the standard, four cues condition. This effect was not seen in control animals.These findings were paralleled by behavioural results. KO animals learned to swim to a hidden platform in a Morris Water Maze task, run with four visual cues surrounding the pool. Their performance was indistinguishable from that of control animals with respect to escape latency, distance travelled, swim velocity and time spent hugging the walls of the swimming pool. In probe trials in which the platform was removed altogether both controls and KO mice spent significantly more time in the target quadrant of the pool. Again, there were no significant differences between the two groups. When a probe trial was run with only one of the four visual cues present, control animals' performance remained intact, while KO animals' behaviour was disrupted. KO animals still showed a selective search strategy in the correct quadrant, but the time spent in this quadrant was significantly shorter when compared to probe trials with the four visual cues present.

Both the electrophysiological and behavioural results described suggest that plastic changes in the entorhinal to CA3 connections and CA3 to CA3 recurrent collaterals are not necessary to generate and maintain place cell signal in CA1, but they are necessary for recalling spatial representations when only some of the cues present during memory encoding are available at the time of recall. The fact that in the single cue condition the spatial selectivity (both at the behavioural and place cell level) is reduced but not completely abolished prompted the authors to suggest that this might be sustained by dentate to CA3 NMDA-independent plasticity and by the direct entorhinal to CA1 projections.

In another study, Brun et al (2002) made ibotenic acid lesions of CA3 and recorded the activity of place cells while rats were foraging in a square enclosure. In a separate group of animals, making razor blade cuts between these two structures and removing the contralateral hippocampus removed CA3 input to CA1. This severed the CA3-to-CA1 connections leaving intact the direct entorhinal to CA1 perforant pathway projections. CA1 place fields were seen in both lesioned and "disconnected" animals. Place fields recorded from disconnected animals were found to have the

same size but significantly higher sparseness and lower peak rates than in control animals and to be stable over days.

At the behavioural level, lesions in the CA3 sub-field led to an impairment in the Morris Water Maze task. Lesioned animals spent significantly less time in the correct quadrant during probe trials when compared to controls. The impairment was only partial, in the sense that CA3 lesioned animals showed a preference for the correct quadrant over the non-correct ones, even though this preference was not as strong as in control animals.

In conclusion both these studies show that CA3 function is not required for the establishment and maintenance of place cells in CA1, and that the direct entorhinal to CA1 projections might sustain spatial behavioural performance although to a reduced level.

Another line of enquiry that might shed some light on the functional relationship between the place subsystems is the anticipatory firing analysis described earlier. Unfortunately the results of such analysis have been rather counterintuitive. For instance subicular place cells have been reported to anticipate the animal future location by a larger amount than CA1 place cells. Anatomical considerations make it highly improbable that the locational signal recorded in the subiculum is fed into the CA1 field (the subiculum strongly receives and only moderately projects back to CA1, see anatomy section). Another rather curious result reported by Taube (1995c) indicates that parasubicular place cells lag behind hippocampal place cells, this again, seems a somewhat counterintuitive result, based on anatomical findings. Timeshift analysis has not been applied to any other area where place cells have been reported.

Head direction cells

General characteristics

Head direction cells were first described by Ranck (1984) in the rat presubiculum. Since then they have been extensively studied and identified in several brain regions. They are predominantly found in brain areas encompassing the classical Papez circuit (Presubiculum, Lateral mammillary nuclei, Anterior thalamic nuclei, latero-dorsal thalamic nuclei), and other limbic areas (restrosplenial cortex). Cells with directional correlates have also been described in portions of the extra-striate cortex (Oc2M and Oc2L) and the dorsal striatum.

Any given head direction cell shows increased firing when the rat points his head in a particular compass direction in the horizontal plane. The rate-direction relationship is well described by a symmetrical triangular function. The angle of maximal firing rate is referred to as the "preferred direction" for that given head direction cell. The firing range is generally defined as the range of head directions over which firing levels are higher than background rates. The signal-to-noise ratio of the head direction signal is very high (70-80) across most of the brain regions where head directions cells have been found.

Unlike place cells, head direction cells never cease firing, in any environment the animal is exposed to. Only when the animal is restrained most of the cells stop firing, resuming activity as soon as the animal is free of moving again (this is true of place cells as well). Another property of the HD system is that whenever multiple HD cells are recorded simultaneously, their preferred directions always stay in register.
An important characteristic of many HD cells is that they anticipate the animal's future heading. The anticipatory interval (the amount of time each HD cell anticipates directional heading) varies across the brain region studied (-6 to 0 msec in presubiculum, 25 msec in ADN and retrosplenial cortex, 40-90 msec in the LMN). There is additional intra-area variability, with each HD cell recorded from the same brain region "tuned" to a particular time-shift.

Firing characteristics of head direction cells in different brain areas

The general characteristics discussed above are common to all the HD cells recorded. Minor differences in peak firing rate, directional firing range and background firing rates have been described for each HD cell population across the brain, although in general reports are not in complete agreement about these parameters. For instance, Blair and Sharp (1995) reported that HD cells recorded from the ADN have higher peak firing rates than presubicular cells. This difference is present in other studies (Taube 1995a; Taube and Muller, 1998), but does not reach statistical significance.

Stackman and Taube (1998) showed that HD cells in the LMN have larger peak firing rates and directional firing ranges than ADN HD cells, a result which is not in agreement with the data presented by Blair et al. (1998). Retrosplenial cortex HD cells have a narrower directional range than the ADN cells (Cho and Sharp,

2001). It is not possible to compare directly the properties of LDN and ADN head direction cells, given that the cells were recorded in completely different conditions (radial arm maze vs open field) and that the techniques used to track the animals heading direction were substantially different in these studies (one vs dual diode directional tracking).

Perhaps a more interesting difference between HD cells across brain areas regards their sensitivity to linear and angular motion.

HD cells from presubiculum, ADN and LMN are all moderately modulated by linear speed, showing increasing firing rates at higher running speeds (Taube, 1998). With regards to angular speed, while the ADN and LMN HD cells firing rate is positively modulated by angular speed presubicular cells are not (Taube et al, 1995a; Taube and Muller, 1998). This subtle difference is very important in light of the models of generation of the head direction signals that will be discussed later.
Importantly, HD cells recorded from LMN show a curious modulation of their firing rate by the direction of head turning. In particular, firing rate increases during ipsiversive turns. So, for LMN HD cells recorded from the right hemisphere, firing rate increases when the rat is moving his head clockwise (Stackman and Taube, 1998). This firing modulation is consistent with the strong inputs that LMN receives directly from the DTN (dorsal tegmental nucleus), and thus indirectly from vestibular nuclei. This effect was not found in LMN HD cells when Blair and Sharp (1998) recorded from them.

Functional relationships between the different components of the head direction system

A series of lesion studies and timeshift analysis have answered many questions related to the functional relationship between the different components of the head direction system.

Lesions of the dorsal presubiculum did not suppress HD activity in the ADN, but severely affected the way in which the HD cell preferred direction changed in response to landmark manipulation (Goodridge and Taube, 1997): that is, HD cells were found to reset between two standard trials, and HD preferred direction did not follow cue card rotation as well as in control animals. These findings suggest that the presubiculum plays a very important role in the HD system, namely it enables HD activity to be reset by environmental landmarks (the ones tested were visual). The presubiculum is indeed ideally placed to channel visual information in the HD system. It receives visual information from several sources, directly from area 18 and indirectly from its afferents from restrosplenial cortex and LDN. It is important to note here that LDN lesions did not suppress HD activity in the presubiculum. Moreover, cue control was preserved in presubicular HD cells following lesions of the LDN (Golob et al, 1998). This suggests that visual information coming from LDN is not necessary to establish cue control in the presubiculum. Lesions in the presubiculum also alter the basic characteristics of ADN HD cells. Both their firing range and anticipatory interval increase, making ADN cells more similar to LMN HD cells. The loss of presubicular inputs thus seems to "uncover" the LMN projections to the anterior thalamus.

Lesions of the ADN cause a complete loss of directional activity in the presubiculum. The effect of ADN lesions on presubicular HD cells fits nicely with the results of the anticipatory analysis conducted on the HD cells of these two structures. Presubicular HD cells code for the current animal heading, while the ADN HD cells anticipate the animals' future location of 25 msec.

Moving down one more step within the HD system, it has been shown that bilateral (but not unilateral) lesions of the LMN suppress HD activity in the ADN. Again, these results are in agreement with the anticipatory firing analysis which showed that LMN cells anticipate the rats' future direction of 40-90msec (Blair and Sharp, 1998; Stackman and Taube, 1998).

Thus the HD signal is probably generated in the LMN (or structures that project to it) and then relayed to the ADN and Presubiculum. Some HD cells have been reported in the DTN, one of the brain areas that more heavily project to the LMN. No lesion studies or anticipatory firing analysis have been conducted on the HD cells of the DTN.

It is not clear how the restrosplenial cortex fits into the HD system. On anatomical grounds, it would be reasonable to suggest that retrosplenial cortex receives HD information from the ADN. Unfortunately no study has been published where retrosplenial HD cells were recorded after lesions in any of the structures associated with HD activity. The somewhat puzzling finding that restrosplenial HD cells anticipate the animals' future head direction by as long as ADN HD cells (25 msec), leaves open the question of whether the HD signal is entering restrosplenial cortex from the LDN.

The possibility exist, therefore, that two distinct HD systems coexist in the rat brain, one originating from deep structures (LMN, DTN), strongly influenced by vestibular inputs, and the other modulated more by visual inputs and originated in the striatal-LDN-restrosplenial areas. Both systems might then converge in the presubiculum.

Head direction cells and landmarks

Several studies have recorded the activity of HD cells before and after landmark manipulations, in an attempt to define to which environmental features the directional system can be tuned.

In general HD cells have been recorded while animals were either foraging in a cylindrical enclosure (identical to the one used for place cell recordings), or performing a working memory task on an 8-arm maze. Both type of environments are placed within a curtained enclosure and have as the sole intentional orientational visual cue a piece of white cardboard which is either taped inside the cylinder or hangs form the ceiling on one side of the curtained environment.

Many studies have shown that when the cue card is rotated the preferred direction of the HD cells recorded shifts a near-equal amount as the cue card. This has been found for all types of HD cells recorded so far, even if the amount of under-

rotation varies across brain region (Taube, 1998). Rotation of the cue card does not affect the firing rate of directional firing range of HD cells.

In a more focused study Goodridge et al (1998) showed that cue card control is a learned property, and that it takes 8 minutes of exposure to a novel cue card for it to act as a polarising stimulus for the HD system. Intervals of 1 or 3 minutes exposure to the card were sufficient to enable it to exert control over about half the HD cells recorded. These results indicate that HD cells can be controlled by a prominent visual stimulus and that, in the appropriate circumstances (generally when the rats are regularly disoriented before entering the curtained area), visual inputs can override the information obtained by other background cues (olfactory, tactile).

Only one study has formally addressed the question of how information by sensory modalities other than visual can modulate the HD response (Goodridge et al, 1998).

In an attempt to test the influence of auditory stimuli on HD cells, rats were exposed to a cylindrical enclosure containing four symmetrically placed speakers. From one of the speakers either a 1-Hz click or white noise was emitted. Rotation of the auditory source did not lead to equal rotations of the HD cells preferred directions. As the authors note, the failure of these auditory stimuli to provide control over HD cells in this paradigm could be due to the symmetric geometry of the cylindrical enclosure. In the same study the authors explored olfactory stimuli, by rotating a prominent olfactory cue (a cotton-bud soaked in peppermint extract) within the cylindrical enclosure. In this case the olfactory cue showed some control over the HD preferred directions, even if the control was short of the one exerted by the visual cue card. Only during half of the recording sessions HD cell preferred direction shifted with the olfactory cues and the under-rotations were both more frequent and bigger than the ones reported in the cue card rotation experiments mentioned above.

The issue of which sensory stimuli can control or affect the spatial firing of HD cells is under-investigated, therefore no firm conclusions can be reached on the issue of whether HD cells, like place cells, are highly "opportunistic" and multimodal in nature.

Head direction cells and darkness

Goodridge et al (1998) showed that when blindfolded rats are placed in a darkened environment, HD cells do not stop firing, and that their preferred direction drifts continuously during the trial. Preferred directions shift around 20-30 degrees between the initial and final 2 min periods of an 8-min session.

These results suggest that in the absence of visual stimuli the HD signal is disrupted, but not suppressed. In a subsequent series of manipulations the authors rotated different portions of the environment (cylinder + floor, only cylinder, only floor) and recorded HD cell activity while the rats were still blindfolded. They showed that both the cylinder + floor and the floor-only rotations caused a similar rotation of the HD preferred directions. This led the authors to the conclusion that olfactory and possibly tactile stimuli attached to the floor were informing the head direction system and affecting HD preferred directions. Thus in the absence of visual

stimuli the HD cells are controlled, albeit not perfectly, by olfactory and tactile stimuli.

The observed drift in preferred directions in darkness is of the order of 30 degrees over an 8 min session. This small drift could be explained in terms of an imperfect path integration system that can only partially be updated by the remaining tactile and olfactory information and slowly accumulates error.

A similar phenomenon has been reported for LDN HD cells (Mizumori and Williams, 1993). In this study HD cell activity was recorded while rats were performing an 8-arms maze working memory task. When the rats were introduced in the maze in complete darkness (without prior disorientation) the "cells fired as the animal moved in many directions in space". If, on the other hand, the darkness period was preceded by as little as 1 min of light, the HD preferred directions were stable for the first 2-3 minutes of darkness, and drifted from then onwards. These results have been taken to mean that LDN HD cells are fundamentally different from the ones in presubiculum or ADN (Taube, 1998), in that they are more reliant on visual stimuli.Given the strong inter-animal variability and sensitivity of the dark trials to uncontrollable extra-maze cues, it is not obvious that the small discrepancies between Goodridge et al (1998) and Mizumori and Williams (1993) results conclusively show that LDN HD cells behave in the dark in a substantially different way from ADN and presubicular HD cells.

Head direction cells and idiothetic information

Given the strong interconnections between the vestibular and the HD systems, it does not come as a surprise that several authors have tried to understand the role idiothetic cues play in establishing and maintaining the HD signal.

Labyrinthectomies (Stackman and Taube, 1997) abolished head direction specific firing in the ADN, showing that vestibular input is crucial for the maintenance of the head direction signal.

Vestibular information is necessary, but not sufficient to maintain stable HD activity. This has been shown by passively transporting rats from a familiar to a novel environment (Taube et al, 1996). HD cells recorded in this paradigm were unable to maintain a constant preferred direction between the familiar and novel environments. It had been previously shown that a stable directional orientation is preserved if rats are allowed to freely locomote between the same familiar and novel environments (Taube and Burton, 1995). Thus self-locomotion derived cues (kinaesthetic, motor, proprioceptive) are required to maintain a consistent directional orientation when the animal enters an unfamiliar environment.

Head direction cells and behaviour

Few studies have addressed the issue of how and if the head direction signal is related to navigational behaviour. This might be due to the specific lack of navigational tasks that could be solved exclusively by using compass-like information. Many of the spatial tasks already available could be solved using a

variety of strategies, some of which are not dependent on directional information per se.

Three studies have studied the relationship between head direction cell activity and spatial behaviour (Mizumori and Williams, 1993; Dudchenko and Taube, 1997; Golob et al, 2001).

In two of the studies rats were trained on a working memory task (8-arm maze), and the activity of HD cells was recorded during acquisition and performance of the task.

Mizumori and Williams (1993) recorded from LDN HD cells and reported that during trials conducted in darkness both spatial performance and HD preferred direction stability was greatly reduced. Moreover, Mizumori and Williams reported that during training, the degree of directionality that HD cells exhibited was negatively correlated with the number of errors the rat made (i.e. the more directional the cell, the fewer errors the rat made). This is in contrast with the results by Dudchenko and Taube (1997). They monitored the activity of HD cells in 4 rats during the acquisition of the radial arm maze task. The preferred directions and directional firing ranges of all the HD cells did not differ at the beginning and the end of the training session. In some of the cells a decrease in the background firing rate was observed. Overall Dudchenko and Taube's results show no indication that the properties of HD cells changed during the acquisition phase of the task.

More importantly, the authors found a strong correlation between animal's arm choice and HD preferred directions. In trials where the cue card was rotated, the animal's arm choice and the HD preferred directions shifted in register. Moreover, when the HD preferred direction did not follow the cue rotation, neither did the animal choose the rotationally correct arm.

Both the Mizumori and Williams (1993) and the Dudchenko and Taube (1997) studies report a strong correlation between HD signal and animal's behaviour, similarly to the place cell study by O'Keefe and Speakman (1987) earlier reviewed.

These studies, being correlational in nature, do not answer the question of whether there is a causal link between HD and place cell activity and animal behaviour.

In an attempt to tackle this issue, Golob et al (2001) conducted an extensive study of the relationship between behaviour and HD cell activity. Rats were trained to go to a corner in a square enclosure for a water reward. The square enclosure was placed in the middle of a curtained environment that had a white cue card attached to one side, as the only intentional polarising cue. Rats were never disoriented during training. The point of entry into the curtained enclosure and the rats' release direction in the square were pseudo-randomised across 16 trials. HD cells activity was recorded after the acquisition phase, in standard and cue card rotation trials. The percentage of correct choices during the standard and cue rotation recording sessions was respectively 77% and 80 % (during cue rotation trials the rats were disoriented before being introduced in the curtained environment).

The main findings of this experiment were that: the preferred directions of the HD cells recorded across standard conditions were less stable than the ones observed when HD cells are recorded in a cylindrical enclosure (only in 77% of the sessions the preferred directions did not vary across standard sessions), and that there was a clear dissociation between rats' behavioural responses and HD cell activity. This was confirmed by the fact that performance was not affected by the HD preferred direction instability (the percentage of correct choices on "stable" vs "unstable" trials was 74% vs 78%), and by the low concordance between HD cell activity and behavioural choice (64%). It is also interesting that the shifts in preferred directions between trials were always multiple of 90 degrees, suggesting that the head direction system was taking into account the four-fold symmetry of the square environment.
HD cells were also recorded when the square enclosure was substituted by a rectangular one. This was done because it had been reported that the preferred direction of HD cells shift between a square and a rectangular arena (Taube et al, 1990). In nearly all the trials (106/114) there were large shifts in HD preferred direction between the rectangle and square baseline. This did not affect performance, with 78% correct choices (vs 77% in the square).

In an additional experiment the authors replicated the findings by Cheng (1986). In the authors' version of the Cheng experiment, rats were trained to go to a specific corner in a rectangular enclosure (sitting in a curtained environment with just one cue card). Rats were disoriented before entering the curtained environment. Similarly to what Cheng found, rats failed to completely acquire the task, showing an inability to distinguish between the two geometrically equivalent corners in the rectangular enclosure and thus ignoring the directional information provided by the cue card. Golob et al recorded HD cells in this paradigm and showed that even though HD cell activity was stable in 56% of the trials, rats' choices were correct only in 36% of the cases.

These results indicate some dissociation between HD cell activity and behaviour in the spatial tasks tested. An important consideration is that these tasks might have been solved using strategies which were not dependent on directional information. Thus, the failure of the HD signal to direct behaviour in these paradigms should not be taken to mean that the HD cell system has no role in spatial navigation.

Models of head direction cells functioning

Several models of how the head direction signal is generated and maintained have been proposed (Skaggs et al, 1995; McNaughton et al 1996; Redish et al, 1996; Sharp et al 1996; Zhang et al, 1996; Blair et al, 1998; Goodridge and Touretzky, 2000). They are all very similar in that they propose that the HD system is organised in an attractor network. Within this network, HD cells are interconnected with both excitatory and inhibitory connections and the geometry of the network ensures that at any given time a "hill" of activity will be generated, corresponding to the one directional heading. When the animal moves his head, the "hill" of activity moves around the HD cell ring, and the directional heading is updated. This is accomplished via asymmetric connections with units which are sensitive to angular velocity. Supporting these models, cells responsive to angular velocity have been described in the LMN and in the DTN (LMN, Blair et al, 1998; Stackman and Taube, 1998; Basset

and Taube, 2001; Sharp et al, 2001b), and the HD cells recorded from LMN are modulated by angular velocity.

To account for the evidence that HD cell activity can be modulated by visual stimulation (cue card rotation experiments), many models postulate that the HD cells also receive environmental inputs. Through experience of any given environment, the connections between the stable sensory inputs and the HD cells become strengthened. Thus, visual (or other sensory) landmarks acquire the ability to control HD cell activity.

The anatomical instantiation of the attractor network is still matter of debate. It was originally proposed that this would reside in the presubiculum. The fact that lesions in the presubiculum do not suppress the directional signal in the ADN, has led other investigators to propose the LMN and DTN as sources of the HD signal (Sharp et al, 1996, 2001).

A problematic issue for all the attractor-type models of HD cell activity is the fact that in the absence of vestibular information HD activity in the ADN is totally suppressed (Stackman and Taube,1997). A prediction shared by all the attractor network models is that the activity "hill" either would freeze in one directional orientation (and this does not seem to be the case) or continually drift along the HD cells ring. Stackman et al (1997) reports bursty activity in the ADN following labyrinthectomies, but says that it does not originate from HD cells.

In contrast to what the models would predict, it seems that vestibular information alone is not capable of updating the head direction system.
Thus it seems that all the complexities of the HD system have not been completely modelled.

Interactions between the head direction and place systems

In the few studies where HD and place cells were simultaneously recorded, their spatial signals have always been strongly coupled (Knierim et al, 1995;1998).
This correlation does not tell us if one system controls the other and to which extent they are parallel. Current indications lead one to conclude that it is more likely for the head direction system to control the hippocampal place system.

In animals with complete dorsal hippocampal lesions it is still possible to record HD cells from the Presubiculum and ADN (Golob and Taube, 1997, 1999). The properties of these HD cells are very similar to those recorded from control animals. Moreover, in hippocampal animals the preferred direction of HD cells in a new environment remains the same during the first and subsequent daily exposures to new environments, and it is subject to cue control.

The converse is not true. In a study which has appeared only in abstract form Archey et al. (1997) showed that after presubicular or ADN lesions place cells recorded from the hippocampus were surprisingly directional, even if the recordings took place in an open field (cylinder). It is also interesting that these lesions frequently disrupted cue control of hippocampal place fields.

In a separate study, Mizumori et al (1994) reversibly inactivated the LDN, showing a profound disruption of both hippocampal place fields and behavioural accuracy on a spatial memory task (radial arm maze).

Chapter V

Theories of Hippocampal function

This chapter will provide a brief account of some of the theories and models associated with hippocampal function.

Both animal studies and findings from neurological patients with damage localised in hippocampal structures show the involvement of the hippocampus in memory and spatial cognition.

The chapter begins with a description of the cognitive map theory proposed by O'Keefe and Nadel (1978), which states that the primary role of the hippocampus is in storing and processing spatial information, and describes some models of spatial navigation. It then goes on to consider theories of hippocampal function which assume that the hippocampus has a broader mnemonic function.

This review will be limited to those theories which seem to be most influential currently.

Cognitive map theory

Immediately after the isolation of place cells by O'Keefe and Dostrovsky (1971), O'Keefe and Nadel began elaborating the theory that the hippocampus acted as a cognitive mapping system (Nadel and O'Keefe, 1974; O'Keefe, 1976; O'Keefe and Nadel, 1978). The following description of the main features of this theory follows the book "The hippocampus as a cognitive map", published in 1978.

One of the major differences between the spatial theory put forward by O'Keefe and Nadel and the prevailing spatial cognition theories of the time, is that the former is based on the conceptualisation of space as an absolute entity (following Kant), while the latters were dominated by a more relativistic view of behavioural and cognitive space.

For O'Keefe and Nadel the hippocampal system generates the organism's intuitive framework of absolute, unitary space. This framework is thought of as innate, and the underlying geometry of the system is Euclidean.

The discovery of place cells (cells which code for the position of the animal in space), prompted the authors to stress the *a priori* nature of space, a space which logically precedes objects, that can exist in the absence of objects.

According to the cognitive map theory, the hippocampus generates and stores cognitive maps of the environments the organism encounters during its life. The map of each environment is composed of a set of place representations (entailed by the

place fields of hippocampal pyramidal cells) connected together by rules that represent the distance and directions among them. We now know that the most likely directional input to the hippocampus comes from the head direction system described in chapter IV. The way distances are computed in the hippocampus is a more debatable subject, but O'Keefe suggests that there may be several ways of measuring distance available to the animal (O'Keefe, 1999). Some of these rely on path integration and, more generally, on self-motion cues, some others could be more visual in nature. (Burgess and O'Keefe, 1996, proposed that an animal could calculate its distance from a wall on the basis of the vertical height, on its retina, of the line where the wall meets the floor). With regard to the self-motion cues, there are preliminary indications that speed might be coded in the hippocampal formation by cells whose firing rate is modulated by the animal's running speed (O'Keefe et al, 1998; Lever et al, 2002b; Sharp, 1996). There are also stronger indications that place cell firing is itself modulated by running speed (Czurco et al, 1999).

O'Keefe and Nadel spelt out the differences between two major systems they believed could support spatial information and processing: a *taxon* and a *locale* system. Only the latter was instantiated by and depended upon the proper functioning of the hippocampus.

The main properties of the *locale* system as opposed to the *taxon* system are that the *locale* system works in an all-or-none basis, and learning within this system occurs in a one-trial fashion, in contrast to the purely incremental learning of the *taxon* system, and that the *locale* system is less prone to interference than the *taxon* one. In O'Keefe and Nadel's view, the hippocampus both constructs and indefinitely stores maps and can do this in an incidental manner. Thus, unlike other neural learning systems, the hippocampus works in an automatic, on-line manner. The hippocampus is also thought to generate and underlie exploratory behaviour.

Though the place cell literature described in a previous section seems to broadly support the cognitive map theory, some of the properties of the place cell system cannot be adequately explained on the basis of this theory alone.

For example, the results of Lever et al (2002) cannot be easily accommodated by the cognitive map theory. This study showed that the changes that occurred in the hippocampal place representations of two geometrically distinct environments were incremental, not all or none and thus were unlike those predicted for the *locale* system by O'Keefe and Nadel. Moreover, the changes were observed at different times in different place cells, showing that the hippocampal cognitive map is not as unitary as originally thought (and see also the results by O'Keefe and Burgess, 1996).
Moving from the animal sphere, O'Keefe and Nadel proposed that in humans the hippocampus has come to provide the basis of episodic memory, through the addition of a linear temporal dimension to the three spatial dimensions of the mapping system. O'Keefe has postulated that the left hippocampus might be specifically dedicated to more linguistic functions and in particular to the generation of narratives (O'Keefe, 1978).

Many behavioural, electrophysiological and lesion studies support the cognitive map theory, and the theory has generated many attempts at modelling in

detail the workings of the hippocampal navigational system. Only a very restricted selection of these will be considered.

Models of navigation

Cognitive Graph Model

The Cognitive Graph model (Muller et al, 1991; Muller et al, 1996; Muller and Stead, 1996) relies crucially on the idea that distances between places are represented entirely by synaptic strength contact between neurons (only CA3 neurons are considered). One of the strengths of the model is that it relies on three basic features: CA3 place cells, an LTP-like process, and dense CA3-CA3 connectivity, which are all well-accepted properties of the hippocampal system.

The authors assume that during random exploration, LTP mechanisms operating at the level of the CA3 recurrent collaterals organise the CA3 network in a "cognitive graph". This is because the strength of contacts between CA3 cells will depend on how close in space their place fields are. During exploration, CA3 place cells with overlapping fields will make stronger contacts with each other, and those with non-overlapping fields will be only weakly connected with each other.

As a consequence of exploration, the connection strength between couples of cells effectively codes for the distance between their place fields. So, if a start and a goal location are defined, the network will easily compute the path of least resistance between them. The graph is also able to sustain detour navigation and short-cuts, provided the animal is given an opportunity to explore the newly opened path (so that new connections will be strengthened between previously weakly connected cells).
The model's successes include the simple demonstration that the network implicitly contains in its structure pseudo-cognitive map behaviour and the fact that the "cognitive graph" is set up in a completely incidental manner, through spontaneous exploration of the environment.

There are some problems with this model. One is that its solution of the short-cut problem requires the animal to explore the new area before being able to use it as a short cut. There is some behavioural evidence that rats learn the allocentric direction to the goal, independently of specific paths (Tolman, 1948; O'Keefe and Nadel, 1978). This is what Tolman referred to as "insight": given several paths from which to choose, the one leading in the direction of the goal would be chosen more often than others, without prior exploration of the paths in question.

Another criticism of the model is that it doesn't include a prediction or description of how the output generated by the graph could be read-out and implemented in a behaviourally significant manner. Which structures support this read-out and how do they accomplish it? Moreover, it is not clear how a goal could be selected (where is the goal represented in neural terms?).

S-R models: Brown and Sharp

Unlike the cognitive graph model, the model proposed by Sharp and colleagues (Brown and Sharp, 1995; Sharp et al, 1996) concentrates on how place cell firing could direct spatial behaviour. It is an attempt at modelling how rats solve the Morris Water Maze task.

Sharp et al have modelled the anterodorsal and presubicular head direction cells as an attractor network, simulating some of the properties observed experimentally within this system. The authors postulate the existence of converging inputs from head direction and place cells within the nucleus accumbens. Place-by-direction cells in the nucleus accumbens are thought of as being connected to motor targets. Learning occurs between the head direction cells and the nucleus accumbens cells.

The major idea underlying this model is that once the rat reaches the reward, recently active synapses are strengthened. This model thus falls into the category of a Stimulus-Response model and therefore suffers from all the criticisms that such models receive. The authors admit that its generalisation power is rather limited. The fact that learning is so reward-driven means that the paths taken towards a goal during training strongly bias all subsequent behavioural responses.
Positive aspects of the model of Sharp and colleagues are that it is simple, and is actually simulated. The architecture consists of sensory cells, place cells, head-direction cells, and motor cells, and the learning rules are conventional.

The models of Burgess, O'Keefe and colleagues

Burgess and colleagues have devoted much effort to modelling details of place field firing and navigation (Burgess et al, 1994; 1996a, 1997; 1998; 2000, 2001; Burgess and O'Keefe, 1996; Hartley et al, 2000; O'Keefe, 1990, 1991; O'Keefe and Burgess, 1996). The model they proposed takes into account several known features of hippocampal formation cell firing, and includes detailed simulations of place fields and their input, the phase shift phenomenon, and head-direction cells and it can also deal with detour behaviour and latent learning (unlike the S-R model described earlier).

The model includes goal cells (assumed to reside in the subiculum), and assumes that whenever a rat encounters a goal location, it turns around to face in several different compass directions. Each goal cell is associated with a conjunction of one definite goal position and a particular head-direction. Goal cells have inputs from the head-direction and reward systems such that the connections from place cells to goal cells can be modified whenever the rat encounters the goal and faces in the appropriate direction. A population vector of a set of goal cells can thus represent the direction to a goal location.

The model relies on the assumption that goal cells exist somewhere in the rodent brain and it makes no attempts at describing how this navigational system is connected to motor structures that execute spatial behaviour.

Hippocampal function beyond spatial cognition

The involvement of the hippocampal formation in mnemonic processing has been known for over 50 years, since the description of the amnesic syndrome in patient H.M. (Scoville and Milner, 1957). The exact nature of this involvement is a highly debated subject, with the main controversies relating to exactly which of the structures comprised within the hippocampal formation are responsible for which type of memories, how long do these structures support memory for (does long-term consolidation occur elsewhere in the brain?) and is this memory function a unitary one or are qualitatively different kinds of memories supported by different structures in the hippocampal formation?

In this section, four types of theory are mentioned: the septo-hippocampal system as a behavioural inhibition system by Gray, the declarative theory of Squire, the multiple trace theory by Nadel and Moskovich and the relational declarative theory of Eichenbaum and Cohen.

The Septo-Hippocampal System (SHS) as a Behavioural Inhibition System (BIS)

Gray (1982)'s theory of the septo-hippocampal system (SHS) did not grant the SHS an important role in memory. Rather, the SHS was hypothesised to be a Behavioural Inhibition System (BIS), its function explained in terms of affect, and anxiety in particular. Fig I.6 presents a cartoon of the inputs and outputs of the BIS as first characterised in Gray (1982). Gray and McNaughton (2000) and McNaughton and Gray (2000) present an updated version of the theory, and this account draws from the latter summary.

An important element of the theory is that the SHS provides a comparator receiving inputs about the state of the world and the expected state of the world. (The original proposal suggested one comparator, located in the subiculum, more recently Gray and McNaughton (2000) have suggested two comparator processes, in the entorhinal cortex and the hippocampus.) This often produces no output, a function characterised as "just checking". When there *is* a "mismatch between the expected and actual events the comparator produces significant output that not only tends to halt the current motor programme but also to cause it to be executed more slowly...or to be replaced by attempts to resolve the problem which gave rise to the mismatch (e.g. increases in exploratory behaviour)" which can "obtain new information to help resolve ... conflict" (McNaughton and Gray, 2000). Theta rhythm is used by the system as a necessary clocking device, helping to maintain the appropriate relationships between sensory and predicted data throughout the different parts of the extended SHS system. Indeed, theta rhythm input from the medial septum defines the elements of the SHS system, including entorhinal cortex, subiculum, and posterior cingulate cortex. Both Gray and O'Keefe then, assign a crucial role to theta.

McNaughton and Gray (2000) identify many similarities between two key experimental manipulations on behaviour: the effects of anxiolytics and the effect of hippocampal lesions, for instance, both *improve* performance on intermittent reinforcement and various active avoidance tasks and *reduce* the amount/performance of spontaneous alternation, rearing, and water maze performance. The pattern of effects is interpreted as suggesting that these two manipulations do not impair motivation, perception or memory in any general sense, and that the bulk of the effects reflect a simple loss of behavioural inhibition.

The comparator concept continues a long tradition of assuming a mismatch role for the hippocampus (O'Keefe and Nadel, 1978; Sokolov, 1963; Squire, 1987; Vinogradova, 2001). In so much as the BIS theory makes an explicit link between the SHS and exploratory behaviour, it is similar to O'Keefe and Nadel (1978) which also posited that the hippocampus was responsible for exploratory behaviour. It could be argued that both theories encounter problems on this account. Consider, for example, rearing, surely an example of exploratory behaviour. Rearing, which in broad terms is reduced both by anxiolytics and hippocampal lesions (McNaughton and Gray, 2000; Harley and Martin, 1999), can be doubly dissociated from hippocampal place cell firing in similar-but-different environments which might be expected to test comparator function; we found that when place cells discriminated between environments, rearing did not, and when rearing discriminated between environments, in most situations, place cells did not (Lever et al, 2003).

In as much as the comparator is often working, yet producing no output, the theory can be compared to the incidental learning stressed by O'Keefe and Nadel (1978) and others and the "automatic recording" by hippocampus of Morris and Frey (1997).

McNaughton and Gray, 2000 interpret the temporal lobe amnesic syndrome as "catastrophic hypermnesia". The loss of behavioural inhibition resulting from impairment of the SHS causes a failure to "suppress storage or recall of incorrect alternatives rather than, as is normally assumed, a failure to store or recall correct alternatives" (McNaughton and Gray, 2000). In other words, McNaughton and Gray (2000) see the hippocampal formation not as producing types of true memories, but aiding the suppression of certain types of false memories. This allows them to account for instance for the presence of intrusion errors in the responses of amnesics (previously correct items produced incorrectly on subsequent trials).

Like many hippocampal system theories, some but not all the experimental data are accounted for. A lot of evidence implicates other areas in novelty detection, such as the perirhinal cortex. Arguably, the comparator theory should delineate in detail the predicted electrophysiological correlates in certain tasks. Why is so much of the input to this system concerned with directional coding (e.g. anterior thalamus and presubiculum)?

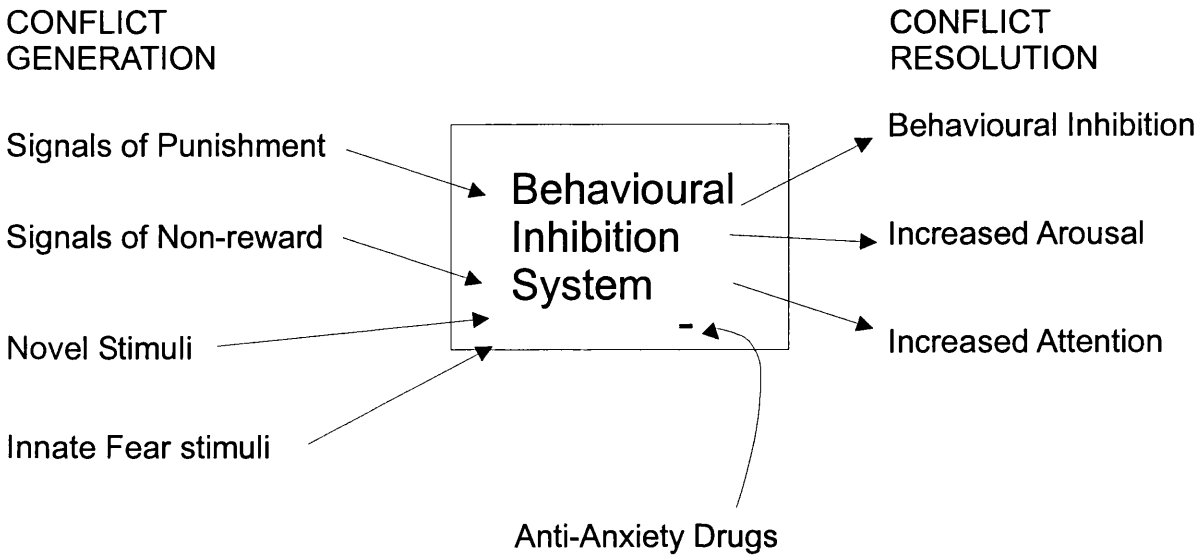


Fig I.6 The Behavioural Inhibition system (BIS) postulated by Gray (1982).
The left hand side shows the classes of stimuli postulated to activate the BIS.
The right hand side shows the outputs of the BIS.
Anti-anxiety drugs act specifically on the BIS.

Adapted from McNaughton and Gray (2000).

Declarative theory

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

Declarative theory recognises the episodic and semantic distinction (Tulving, 1984), with episodic memory relating to events and semantic memory to the retention of factual knowledge. The declarative theory predicts that the medial temporal lobes are involved in both types of memory and that the extent of the memory impairment varies directly with the amount of tissue damaged. Accordingly, studies that suggest purely episodic deficits, with preserved semantic learning, for patients with bilateral hippocampal damage, are problematic for the declarative theory (Vargha-Khadem et al, 1997) and are interpreted within the theory as not providing real dissociations between the semantic and episodic realms.

With regards to the animal literature, the vast majority of studies using restricted ibotenic acid lesions of hippocampus have failed to support a role for the hippocampus in non-match to sample tasks and recognition memory tasks (eg. see references in Jarrard, 1993; Mumby et al, 1996; O'Keefe, 1993). Furthermore, Mumby et al, (1996) helped to explain previous recognition deficits associated with ischaemic hippocampal lesions (Zola-Morgan et al, 1986; 1992) by showing that ischaemic-induced hippocampal rats showed recognition memory deficits but when their hippocampi were removed after ischaemia, their performance was unimpaired.
The issue remains somewhat controversial and Squire and collaborators have more recently reinvestigated this issue in monkeys, and re-analysed old data, to suggest that damage limited to hippocampal region (CA fields, dentate gyrus, and subiculum) impair recognition memory at longer delays (Zola et al, 2000). Nadel (eg. 1994) has pointed out that during the longer delays some of the animals in this recent re-analysis were taken to another room, and then returned, and a failure to recognise the original testing context may be partly responsible for the deficit.

One of the other features of this theory is its prediction about consolidation. The theory has arguably been more successful here. Squire's theory, based on the clinical literature on retrograde amnesia, has emphasised the idea that hippocampal storage is time-limited and that neocortical areas gradually acquire memory traces stored in hippocampus. In essence, memories which were dependent on the hippocampal formation can become a neocortical-dependent, hippocampal-independent memories. Two one-subject studies have recently shown intact remote spatial memory in patients with profound hippocampal damage (Teng and Squire, 1999; Rosenbaum et al, 2000). Although, it is possible that the spatial learning acquired by these patients is not cognitive-map like, and that parietal and frontal cortex for instance can support some of the learning tapped by the tasks used to assess spatial layout knowledge these two papers undermine the view that the hippocampus is required at all times to store and retrieve spatial memories.

Multiple trace theory

Nadel and Moscovitch (1997) drew evidence from the animal literature, amnesic patients studies and neuroimaging studies in healthy subjects to support the opposite view that certain types of memories are encoded and permanently stored within the hippocampus. According to their account of hippocampal processing, the hippocampus automatically encodes memories, acting as a pointer, or index to those neurons in the neocortex that represent the sensory cues and other features of the attended event (Teyler and DiScenna, 1986). Thus, in order to reactivate a memory one requires an intact hippocampus. When a memory is replayed, in a different temporo-spatial and neural context a new trace is laid down in the hippocampus. This means that over time, some memories get strengthened through rehearsal and are represented by several traces in the hippocampus, while some others are lost. Thus, the evidence of a graded retrograde amnesia that the consolidation theory holds onto to demonstrate that memories, once "mature", are shuttled to the cortex, could be reinterpreted on the basis that partial hippocampal lesions could selectively spare well-rehearsed memories as opposed to more recent memories which are encoded in fewer hippocampal traces.

In support of Nadel and Moskovitch's views, several neuroimaging studies in healthy subjects have showed that activation of the hippocampus is as robust when remote memories are being retrieved as when recent memories are retrieved (Ryan et al 2001; Maguire 2001). These findings speak in favour of a permanent hippocampal role in memory retrieval.

Another important feature of the multiple trace theory is that the hippocampus is thought to be involved in the processing of only a subset of mnemonic material. For Nadel and Moskovitch the hippocampus is the seat of episodic memories and in particular of autobiographical episodic material, while they think that more semantic material, like factual knowledge about the world and the self might become, over time, hippocampally-independent.
Studies on amnesic patients seem to support this interpretation, given that the retrograde amnesia for autobiographical events, when tested, extends over very long periods of the subjects' life (30 years or over), and it shows, in general, a rather flat gradient (the deficit being similar in intensity at all time periods considered), while

semantic information is generally more mildly affected and shows a more graded retrograde amnesia.

Additional support for the idea that the hippocampus might support very selective mnemonic material comes from the animal literature, where several studies have shown that when the animals are tested in spatial tasks, lesions in the hippocampus induce a dramatic and flat retrograde amnesia. On the contrary, when the animals are tested on non spatial tasks (object discrimination or others), and then hippocampal lesions are induced, one sees either no retrograde amnesia or a graded amnesia (Zola-Morgan and Squire, 1990; Salmon et al, 1985).

Relational Declarative theory

Eichenbaum and colleagues have emphasised that the hippocampal system supports a relational representation of items in memory, and that these representations are flexibly stored within the hippocampus, allowing the organism to use them in an inferential way, in novel situations (Eichenbaum, 1994). The relational theory of hippocampal function considers the cognitive map theory of O'Keefe and Nadel only a special case of a more general relational learning function of the hippocampus.

The theory rests on some electrophysiological and behavioural evidence.
As for the electrophysiological evidence, this is based on hippocampal place and theta cells recording in rats subjected to several behavioural paradigms, which have been taken to support the view that place cells do not code just for spatial cues, but have a more general role in coding for elemental non-spatial stimuli and, more crucially, conjunctions of stimuli (as the relational theory predicts that the hippocampus is required in encoding "relations" among simple stimuli). It has been pointed out that several of these secondary properties of place cells might be observed in the tasks used because such tasks introduce intrinsic spatial confounds (O'Keefe; 1999; for example, in almost all the tasks used, the animal is required to move along stereotyped routes and thus most of the approach-to-goal responses observed in the hippocampus might reflect the activity of place cells which have developed directional place fields under these behavioural circumstances).

These general criticisms, do not apply to a very well controlled experiment conducted by Wood et al (1999), where the rats were required to run a continuous olfactory non-match to sample task. Hippocampal cells were recorded and separated into theta and complex spike cells on the basis of their waveform characteristics, and the activity of both classes of cells statistically analysed in the 1-sec long time window which preceded the animal's choice. 91 cells out of 127 recorded had statistically significant correlates. 40 cells (31.5%) had spatial correlates (either only position or conjunction of position and odour, position and match/non-match or position and match/non-match and odour), while 51 cells (40.2%) had non-spatial correlates. 26 of the non-spatial cells were classified as approach cells, because they showed an increased firing whenever the rat approached a sand pot, regardless of the trial type, position or odour of the pot. There were 10 odour cells, 13 match/non-match cells and 2 odour and match/non-match cells. According to the relational theory of hippocampal function the hippocampus encodes the conjunctions of stimuli relevant for task acquisition. Given that the task used by Wood et al is an olfactory non-match to sample task, and that performance during recording was 96.8% correct,

it seems surprising that only two cells were encoding the odour and match/non-match relationships and that many cells were coding for elemental odour or position cues. Moreover, the fact that 31.5% of the cells showed spatial correlates, given that position was not one of the relevant variables for task acquisition, is not predicted by the relational theory.

To support the relational theory Eichenbaum and colleagues have performed behavioural studies which indicated that the hippocampus might have a role in non-spatial tasks. One of them is the social trasmission of food preference (Bunsey and Eichenbaum, 1995). This is a non-spatial associative task which consists of three stages. In stage one a demonstrator rat eats a scented food. In stage two the demonstrator comes into contact with an observer rat. The observer rat forms a non-spatial association between two stimuli present in the demonstrator rats' breadth (the odour of the food that was eaten by the demonstrator in stage one and carbon disulphide; Galef et al, 1988). In stage three the observer rat is given the choice of eating two scented foods, one of which is the one previously eaten by the demonstrator. The observer rat will preferentially eat the demonstrator's food. The food preference is retained after a 24 hrs delay after the demonstrator-observer interaction.

Bunsey and Eichenbaum (1995) showed that animals with ibotenic acid lesions of both hippocampus and subiculum exhibited a significant memory deficit on this task after the 24 hr delay, while animals with lesions restricted to the hippocampus or the subiculum did not show any deficit. Burton et al (2000) failed to replicate these findings, despite considerable effort in trying to mimic as closely as possible the procedures used in the Bunsey and Eichenbaum's study. Given that the nature and the extent of the lesions were very similar in the two studies, it is very difficult to reconcile these two contradictory findings. Thus, it remains unclear whether this task depends on the integrity of the hippocampal-subicular complex.
In conclusion, the claim that the hippocampus is involved in encoding relational associations needs further investigation.

Methods

Chapter VI

Subjects

In total, 14 male Lister hooded rats were used in the experiments described in this thesis. Rats weighed between 280 and 400g at time of surgery, were housed singly in Perspex cages, and maintained on a 12:12 hour light:dark schedule, with lights off at 3 pm. Rats were weighed daily, and maintained at about 90% of their free feeding weight. This was sufficient to ensure motivation for the behavioural requirements of the experiment.
Rats were implanted with electrodes before the various experiments and no pre-implant selection procedure was used.

Electrode construction

The microelectrodes used were constructed from HM-L coated platinum-iridium wire (90%/10%; California Fine Wire) either 17 or 25 μm in diameter (no consistent differences in results were observed between the 17 and 25 μm electrodes). The electrodes used were either stereotrodes (rats A and H) or tetrodes (all the other rats).

Stereotrodes and tetrodes were constructed by twisting respectively two or four individual fine pieces of wire together. For stereotrodes this was done by cutting an appropriate length of single wire, sticking the ends together into one loop which is then hung on a post and the two wires were twisted clockwise (about 15-20 turns per mm). For tetrodes a similar procedure was used, but after sticking together the wires in a loop this was bent on itself to form a second loop, then the two loops were hung on a post and the four wires were twisted together clockwise. The end of the loops was cut to form two (stereotrodes) or four (tetrodes) free ends. The HM-L insulation of these ends was then removed using an alcohol flame and the bare wire was tied to the posts of the drive. The other end was cut to form the stereo/tetrode tips which were then implanted into the brain.

Microdrives

The electrodes were loaded into a microdrive apparatus shown in fig VI.1.
11 out of 15 animals were implanted with the standard 8-channel microdrive which accommodates up to 8 wires (2 tetrodes or 4 steretrodes), 3 animals (rats N, E and F) were implanted with 16-channel microdrives (4 tetrodes), and one animal was implanted with a 32-channel microdrive (8 tetrodes). In one case (rat D) 4 tetrodes were implanted using two 8-channel microdrives with two tetrodes each *in each hemisphere.*

Both the 8- and the 16-channel drives do not allow the independent movement of the electrodes, the 32-channel drive allows the independent movement of each of the 8 tetrodes.

8- and 16-channel drive description

Figure VI.1 is a diagram of the standard 8-channel microdrive, showing its mechanical features. This apparatus consists of a stationary part composed of two metal posts. These two posts are joined at the bottom (soldering joint near the word "frame" in figure VI.1) and at the top (with a flange and a nut). One of the posts (right hand side in figure VI.1) carries a fine-threaded hollow screw. The dimensions of these two tubes are chosen so that the second tube may turn over the second post without wobbling. Two small sections of heatshrink tubing are fitted over the screw-thread and over the second post and heated. The heatshrink tubing over the screw-thread becomes moulded to the thread in the process, thus forming a "nut". Dental cement is then applied around both these pieces of tubing. This forms the basis of the moveable part of the microdrive. Not shown are the plug and connection wires mounted onto this moveable part of the microdrive using dental cement. The standard setup is to mount 8 wires, 4 on each side, onto the moveable part. However, 16 wires are mounted in the case of 16-channel microdrives. The connection wires are soldered at the plug end, so that electrical signals in the wires can reach the headstage, when the headstage is connected to the plugs. At the other end, the connection wires are stripped of insulation to form posts and a short length of 23ga (or 21ga for 16-channel drives) stainless-steel cannula is mounted on the front between these posts using dental cement. A piece of 19ga (or 17ga for 16-channel microdrives) tubing is cut sufficiently long to cover the length of cannula protruding from its cement mounting and is slid over this ("sleeve" in fig VI.1; in the figure the sleeve is shown in its final position, after the electrodes are lowered in the brain during surgery). The tetrodes are mounted by sliding them through the cannula from the top, leaving a predeterminate length protruding from the far side. The bare ends of the tetrodes protruding from the top of the cannula are wound round the connection posts and then painted with electrically-conductive paint.

The microdrive/electrode assembly is then sealed using non-conductive nail varnish. Many thin coats are applied, to prevent the inclusion of gas pockets in the drying varnish.

When the feet of the drive are fixed in space, turning the screw will cause the moveable part of the microdrive to move up or down. The screw is turned by a "screw-turner" (fig VI.1) mounted round the post above the screw. Accuracy is ensured by the "spring" which maintains contact of the lower screw bearing surface against a fixed "collar", with sufficient pressure to prevent vertical movement. A 360° turn of the screw moves the cannula and electrodes down by 200 μm. In general no turn is made of less than 45 ° (ie 25 μm).

During surgery the feet of the drive, and the sleeve around the cannula encircling the electrodes, are affixed to the skull using dental cement.

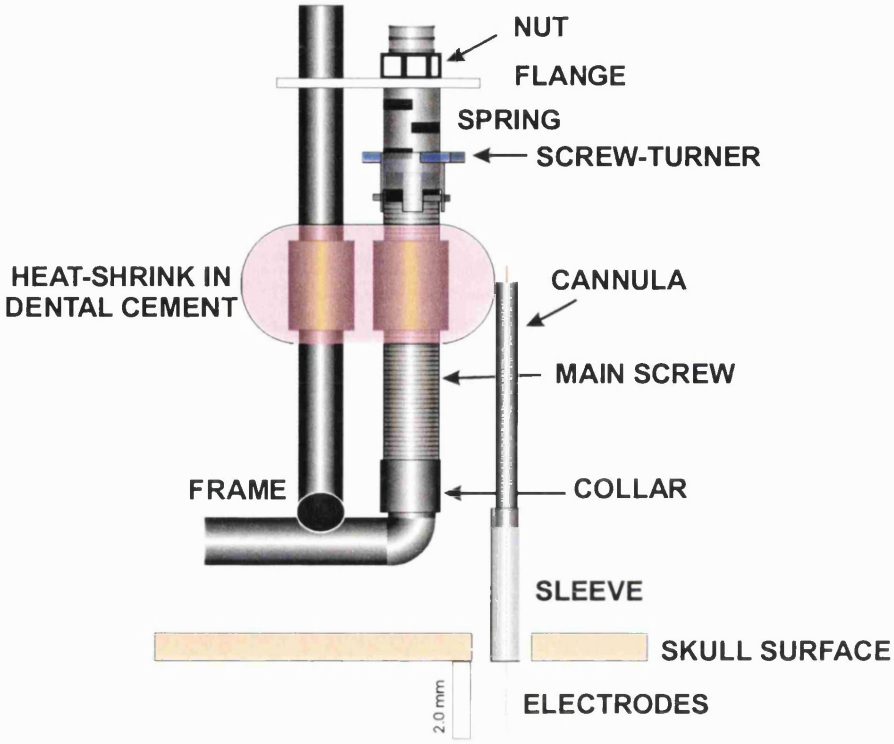


Fig VI.1This diagram shows the main features of the microdrive in the stage before dental cement is applied to connect the cannula to the main screw. Once this is done, turning the screw-turner causes the cannula to move up and down relative to the main screw. At implant, the electrodes might be implanted, as shown here, about 2.0 mm below the skull surface.

The microdrive was loaded with up to four tetrodes.
(This figure was originally drawn by John Huxter.)

32-channel microdrive description

The 32-channel microdrive can be loaded with up to 8 tetrodes. The important difference between this large drive and the ones described above is that in this case the drive is made of 8 separate screws, *this allows for independent movement of each tetrode*. The drive comprises 8 posts arranged in a circle. The electrodes fixed to each screw are all fed, and move, through a circular array of individual cannulae. At the bottom of the drive, the tetrodes fit into a sleeve, which resembles an 8-bullet gun revolver. As with the drives described before, during surgery dental cement is used to fix the feet of the drive and the sleeve to the skull.

Surgery

The animals were removed from the animal house and placed in individual cages in a holding room close to the operating theatre at least one day prior to surgery. After surgery, the animals were kept in this room until they were killed at the end of the experiment.

The animals were anaesthetised using isofluorane and nitrous oxide, and given an i.m. injection of buprenorphine (45μg) for intra- and postoperative analgesia, and an s.c. injection of enrofloxacin (2.5mg) as a prophylactic antibiotic.

The animals were first anaesthetised using the gas preparation as follows: oxygen at 3 litres per minute, with isofluorane at 3% of the gas volume. The animal's head was shaved using electric clippers. Before the animal was fitted with the ear bars, nitrous oxide was introduced at 3 litres per minute, and the oxygen flow was reduced to 1.5 litres per minute. Around this point, the i.m. injection of buprenorphine and s.c. injection of enrofloxacin were administered. The percentage of isofluorane was gradually decreased throughout surgery, and usually ended at levels of 0.5 to 1.2 %.

Once the animal was fitted with ear bars it was mounted in the stereotaxic frame. The skull was exposed and the rat's head adjusted so that the upper surface of the skull was parallel to the base plate of the stereotaxic frame.
Seven holes for screws were drilled using a 1.2 mm burr drill. Two holes were drilled over the occipital, two over the frontal bone, one on each side in both cases. Two further holes were drilled over the parietal bone, contralaterally to the implant side, and one over the parietal bone rostral to the implant site. Stainless steel screws were screwed into the seven holes. One screw, soldered with a gold amphenol pin, was screwed into the right frontal hole and served as the ground attachment. Another hole for the electrodes was drilled using a 2.3 mm trephine drill above the target coordinates.

The pial surface of the brain was then exposed, removing the dura mater using the tip of a 30ga needle.

The microdrive-tetrode assembly was then stereotaxically positioned with the electrode tip at the target Bregma coordinates, and then lowered so that the electrodes were placed at the appropriate depth below the pial surface of the brain.
The sleeve around the electrode cannula was then lowered so that its lower surface rested on the brain surface. In this position, the sleeve covered the length of exposed electrode between the bottom of the cannula and the brain surface. The exposed brain around the sleeve was loosely packed with haemostatic sponge.
The tetrode-microdrive assembly was then fixed to the skull by applying dental cement around the sleeve, the feet of the microdrive, the screws and the skull.
A plastic screw, screw end down, is cemented to the skull, providing the attachment for the LED-holder part of the headstage. Other plastic screws are also cemented where necessary to protect the drive from any grooming movements by the animal.

Unit recording

Screening

Approximately one week after surgery, recording began. Screening for electrical activity took place while the rat was on the holding platform. While cortical electrical activity could be detected (judging from the amplitude of the signals and the calculated position of the electrodes) the electrodes were advanced in 50 μm vertical increments each day. When cortical activity disappeared the electrodes were slowly advanced in the brain in 25 μm steps each day for several weeks.

Electrical recording apparatus

Each rat was connected to the recording equipment via a headstage which fitted onto the plug of the microdrive. The headstages used were unity-gain buffer amplifiers; the implanted electrodes were AC-coupled to these amplifiers, which served to isolate the electrodes from the wires carrying their signals to the recording system. Lightweight hearing-aid wires 2 to 3 metres in length connected the headstage to a preamplifier (gain 1000). The outputs of the latter passed through a switching matrix, and then to the filters and amplifiers of the recording system. The switching matrix is an array of analogue switches to which all of the electrode signal inputs and all the amplifier boards are connected. Under software control, the switching matrix allows the user to control which signals get recorded differentially with respect to which other signals.

Signals were amplified (c. 15-50 thousand times) and bandpass filtered (500 Hz-7 kHz). Each of the four channels of a given tetrode was recorded differentially with respect to a channel on another tetrode. Differential recording is now a standard technique, used to subtract noise and artefact from the signal. As the tips of the tetrodes (or stereotrodes) were generally separated about 200 to 500 μm, differential recording served to remove common noise while preserving action potentials close to one or other of the tetrodes (stereotrodes). Each channel was continuously monitored at 20-μs intervals (sampling rate 50 kHz) and potentials were captured with 50 sampling points per channel (1ms, with 200 μs pre-threshold and 800 μs post-

threshold) whenever the signal from any of the pre-specified recording channels exceeded a given threshold set by the experimenter (ie when a presumptive neuronal spike occurred).

Two small, infrared light-emitting diodes (LEDs) were attached to the rat (anchored via a plastic screw cemented to the animals' skull during surgery) for the purpose of tracking the rats' head position and orientation with a video camera and position-detection hardware (position sampling rate 50Hz). The two LEDs were separated by 5-7 cm and identified on the basis of their differential brightness. Extra care was taken to ensure that the position of the LEDs on the animals' head remained stable during the whole experimental procedure. Given a pixel size of 6.1 cm^2 and a distance between the two lights of minimum 5 cm, the minimum angle that could be resolved was 8°.

Each spike event was spatiotemporally stamped with: a) the time since the start of the recording, and b) the x, y location of the LEDs, as determined by the position-detecting hardware.

The EEG signal from the animal was obtained from one wire of one of the implanted tetrodes. Like the spike signals, it was buffered by the unity-gain buffer amplifiers in the headstage before passing along hearing-aid wires to a preamplifier, where it was amplified 1000 times. The signal was then low-pass filtered at 500 Hz and notch filtered at 50 Hz to remove mains frequency noise, and amplified further, typically by a factor of 15. Like the spike signals, the EEG was initially digitised at 48kHz, then routed to a digital signal processor where it was anti-alias filtered in software at 125 Hz and down-sampled to 250 Hz. The 250 Hz samples were stored along with the position data (five EEG samples per position) on the hard disk.

At the end of a recording period, all the data were stored on a hard drive and transferred to a SUN workstation, for later analysis.

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

Laboratory layout and behavioural task

The experiments described in this thesis were conducted in two separate (although very similar) laboratories. The general laboratory layout is given in fig VI.2. In all the experiments recording took place while the rat was foraging for sweetened rice grains scattered randomly by the experimenter in differently shaped environments. This behavioural task is very similar to the pellet-chasing paradigm developed by Muller and Kubie (1987) and used by many others since. Rice throwing was not done entirely pseudo-randomly but was sensitive, especially later on in the trial, to the locations the rat had already visited, in the interest of homogeneous coverage of the environment.

Light protocol

Recording took place in differently shaped environments placed within or outside the cue controlled environment (CCE).
Outside the CCE cellular activity was recorded while the rat chased rice on the holding platform, a square, unbounded wooden platform raised 80 cm from the floor.
Inside the CCE the recording environments used were:

a) a black, unbounded circular platform of diameter 90 cm, raised from the floor;
b) a grey cylindrical enclosure, mounted on the black circular platform (diameter 79 cm, height of the walls 51 cm);
c) a grey square enclosure, mounted on the same black circular platform (side-length 62 cm, height of the walls 51 cm).

In some experiments other environments were used, they will be described in the relevant section.

The animals were brought in and out of the laboratory and the environments according to standard procedures. At the beginning of any given day the rat was brought into the laboratory and placed on the holding platform, placed outside the curtained enclosure (fig. VI.2). At the beginning of each trial the rat was carried inside the curtained environment with its head always facing laboratory north, through an opening in the south side of the black curtains. The rat was then placed in the middle of the given walled or unbounded environment. The intertrial interval varied between 10-30 min.

Dark protocol

The environment used for the dark experiments is the cylindrical enclosure described before, mounted on the black circular platform. This was in turn mounted on a rotating stool. No cue card was present inside the curtained enclosure and four lights were arranged symmetrically, hanging from the ceiling of the curtained enclosure.

The lights and the speakers' audio output were turned off around 2 min before the recording session began. The experimenter wore infrared goggles throughout the dark period to perform the manipulations required. The rat was brought inside the curtained enclosure as described before and placed inside the cylindrical environment, and was thus not disoriented.
The first recording session lasted for 20 minutes.
The second recording session began a couple of minutes after the first finished, to allow for the transfer of the data from the hard drive to the SUN workstation, and it lasted for 14 minutes.

Four minutes after the second session began the lights within the curtained enclosure were turned on and the rat kept exploring the now lit environment for an extra 10 minutes.

In some cases this protocol failed to induce the drift in the head direction system and a variant of the experiment described above was carried out on a different day.

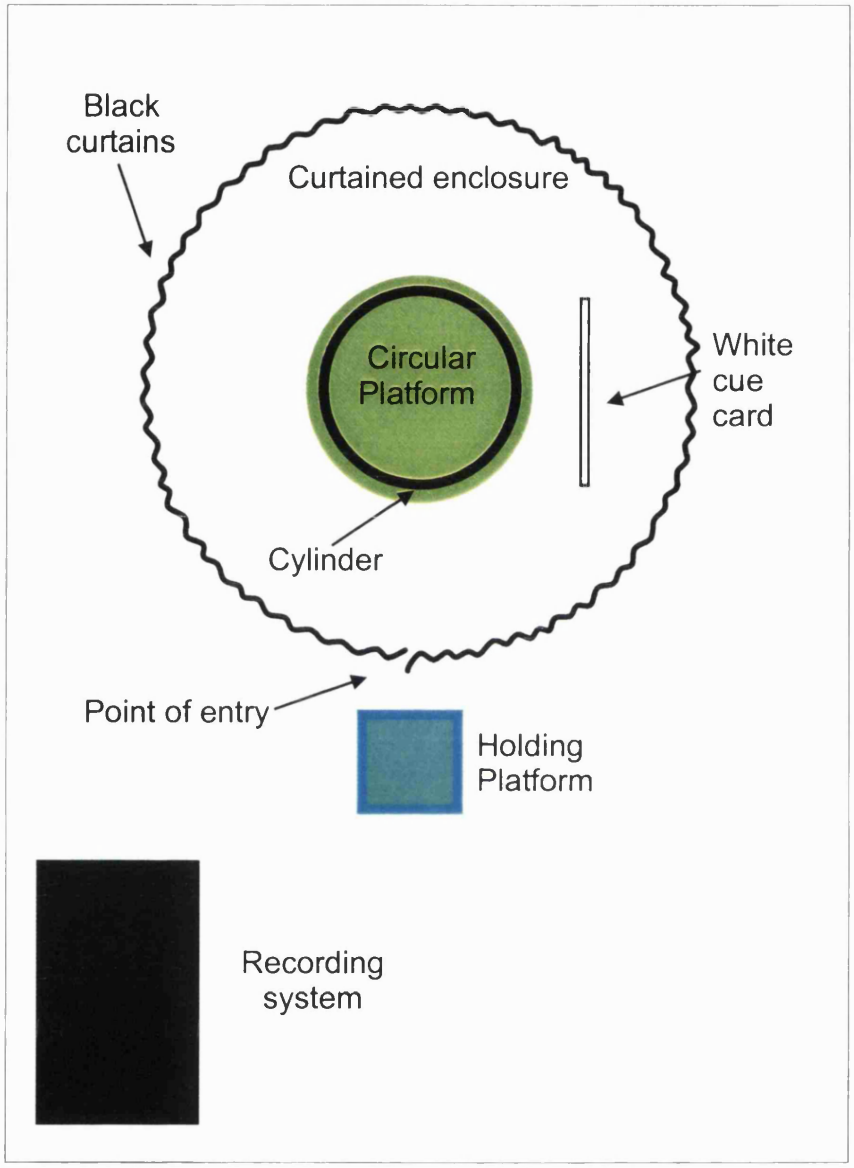


Fig VI.2 This diagram shows the typical laboratory set up, configured for recording in the Cylinder (also referred to as the Circle).

The Cylinder (black circle) can be *removed*, to leave the Circular Platform (green) open to the curtained environment.

The Cylinder can be *replaced* by the Square-walled box (not shown), made of the same wooden material and painted in the same way.

Recording can also take place outside the curtained environment entirely, on the square Holding Platform shown (blue).

.

Five minutes after recording began, the rat was picked up and lifted from the floor for around 10 seconds, without being rotated or horizontally displaced. At ten minutes during the recording session the rat was picked up again, lifted from the floor, and the floor and the cylinder were quickly rotated clockwise of 180 degrees. The rat was put back on the floor and allowed to continue foraging for other 10 minutes.
The second recording session was identical to the protocol described before. Between the first and second recording sessions the rat was left into the cylindrical environment while the experimenter kept throwing rice grains.

Data analysis

Cluster cutting

The process of isolating single units from the originally multi-unit recording is called cluster cutting.

For all the data used in this thesis cluster cutting was performed manually on SUN Ultra workstations and computers emulating UNIX systems, using custom made software (TINT, written by Neil Burgess). No automatic cluster cutting algorithms were used.

Collected waveforms were plotted and separated into clusters on the basis of peak-to-peak amplitudes, and waveform shape (strictly speaking, voltage heights at particular times).

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

Derivation of firing rate maps

Derivation of place fields

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

The camera viewing area was divided into a 64x64 bin grid system. The camera was an analogue camera, with digitisation performed by the tracker at 10 bit resolution. Thus its spatial view is divided into a theoretical maximum of 1024 x 1024 spatial units. In practice, the total possible camera viewing area is 768 pixels in the x dimension, and 574 pixels in the y dimension. The analysis software (TINT) places all coordinate frames less than or equal to 512 x 512 pixels within a 512 x 512 viewing area. It is this "camera viewing area" that is divided into 64 x 64 bins. The camera viewing area was scaled so that the size of the different environments would appear the same on the TINT window. This was done to ensure that the binning would be consistent across data acquired in different laboratories. In lab A, for instance, the pixel size is 2.9 mm, in lab B the pixel size is 4.0 mm for the platform raised 26 cm from the floor, and 4.5 mm when the platform was mounted on the rotating stool for the dark protocol (raised 55 cm from the floor). Accordingly 1 bin will contain 8 pixels in lab A, and 5.8 and 5.2 pixels in lab B respectively for the light and dark conditions. An important consideration is that any given environment will include a fixed number of bins, regardless of the experimental conditions. The cylinder's diameter is always 34 bins long, the square's side is 25 bins.

The basic procedure for deriving firing rate maps is that for a given bin, the number of spikes of each cell in that bin is divided by the rat's dwell time in that bin, to give a firing rate for each cell in that bin. However, following previous work (O'Keefe and Burgess, 1996; Jeffery and O'Keefe, 1999), rather than display the data bin-by-bin, a box-car averaging, or 'smoothing', is applied to the map. This is in order to compensate for the poor sampling of places that sometimes occurs during recording. When smoothing is applied, the rate of a particular bin is derived from the total number of spikes fired in that bin and surrounding bins, divided by the total dwell time in that bin and the same surrounding bins. The number of surrounding bins used is determined by the 'level' of smoothing. The level used in this thesis is 5 in the TINT program. Smoothing level 5 means that for each bin, a larger square with 5-bin-long sides, whose centre is the current bin, is used to smooth the rate of that bin. This smoothing is applied to every bin in the environment, ie. the larger, 25-bin-in-area, smoothing squares are overlapping. Only visited bins are considered during smoothing, therefore at the edge of the environment, less than 25 bins would be used.

The firing rate in each bin is mapped as a colour or grey-scale plot. The 5 colours or shades are autoscaled so that each represents 20% of the peak rate. In descending order, bins with the highest rates are shown in red, then yellow, then

green, then light blue, then dark blue for the lowest rates. A white bin represents an area that is unvisited.

Derivation of polar plots

The directional firing characteristics of both HD and TD cells are displayed as polar plots. The directional range (0-360°) is subdivided into 36 bins of 10° each. As for the place maps, the number of spikes that the cell fired when the animal was facing its head in a specific direction is divided by the time spent facing that direction. No smoothing is applied.

A polar plot is drawn, where the angular orientation of the head is represented by a circular grid (laboratory north equals 90°, east is 0° in these plots), and the firing rate of any given head orientation is represented by the distance from the centre of the grid (bigger distance means higher firing rate). Each point is then connected to form a closed line.

The preferred direction of a cell is the direction at which firing rate is maximal. Directional firing range is calculated as the extent of arc in which firing is half the maximal firing rate.

Autocorrelograms

The auto-correlation function of a spike train gives the frequency of occurrence of a pair of spikes separated by a time τ.

$$\phi(t) = \lim_{T \to \infty} \frac{1}{2T} \int_{-T}^{T} f(t).f(t+\tau)dt.$$

The autocorrelogram is a histogram formed by dividing the range of τ into equal bins centred on τ_i and counting the number of spikes whose temporal separation falls into each bin, and it can be thought of as an approximation of $\phi(t)$. The autocorrelogram is then normalised by the number of pairs of spikes observed during the trial to give the probability density function for the time between the spikes. For all the autocorrelograms shown in this thesis the time bin is 50 ms.

EEG fitting and phase preference analysis

Topodirectional cells show a tendency to fire at particular phases of the locally recorded theta rhythm. The EEG phase at which spikes were fired was determined by fitting the EEG data to a sequence of half-sinusoids. The maximum mean square error allowed for fitting a theta cycle was set to 24, and the minimum time allowed between fitted sinusoids to successive theta cycles was 0.08 s.

All the spikes for which no theta fitting was possible were discarded. Only cells for which at least 50% of the total number of spikes fired had a theta phase assigned were included in the phase preference analysis.

Waveform analysis

During recording, whenever the voltage exceeds an experimenter-set threshold value, a 1-ms long signal trace is recorded. This 1-ms long trace encompasses 200 μs before and 800 μs after the time the threshold was crossed. This signal trace is sampled at 48 KHz (i.e. each 1-ms trace is composed of 48 data points).
Thus, for each cell isolated (see cluster cutting) a representative waveform is constructed by averaging the traces of all the spikes collected for that given cell. For this averaged waveform, two parameters are measured: 1) the difference between the maximum (peak) and minimum (trough) voltages (peak-to-trough amplitude), 2) the time between the peak and the trough.

Information measures

Several measures were computed to quantify the spatiality of topodirectional cells firing and to compare it to that of place and head direction cells.
Information content was computed for both the place and directional components of the cells' firing, using the spatial information measure introduced by Skaggs et al (1993) and a modified version of this method developed by Neil Burgess (Burgess et al, in preparation). They measure the amount of spatial information (be it locational or directional) carried by each spike the cell fires, expressing it in bits per spike.
Briefly the estimate of the rate of information I(R|X) between firing rate R and location X proposed by Skaggs et al (1993) is:

$$I(R \mid X) \approx \sum_i p(\vec{x}_i) f(\vec{x}_i) \log_2 \left(\frac{f(\vec{x}_i)}{F} \right)$$

where $p(\vec{x}_i)$ is the probability for the rat being at location $\vec{x}_i$, $f(\vec{x}_i)$ is the firing rate observed at $\vec{x}_i$, and F is the overall firing rate of the cell.
There is an intrinsic problem with the estimation of the spatial information carried by each spike the cell fires: the data acquired is strongly under-sampled, and we generally can only produce an estimate of the firing rate of the cell in any given bin (estimated here as $f(\vec{x}_i)$, the mean firing rate observed at bin $\vec{x}_i$). There is not enough data to properly estimate the reliability of the observed $f(\vec{x}_i)$. The Burgess information measure is an attempt at tackling this problem, by assuming that the probability distribution of firing rate in bin $\vec{x}_i$ follows a Poisson distribution with its mean equal to the observed rate $f(\vec{x}_i)$.

The information measures described above do not capture the spatial structure of the signal analysed. They do not take into account how firing rate changes across spatially contiguous bins. As a measure of the spatial orderliness or "compactness" of the locational firing of each cell, the spatial coherence measure was computed, using the procedures of Kubie et al (1990). This measure has the disadvantage that it quickly goes to ceiling if the spatial data considered is smoothed. So, in the case of smoothed data an alternative measure was used. This is based on a spatial autocorrelation method, introduced by Neil Burgess (Burgess et al, in preparation),

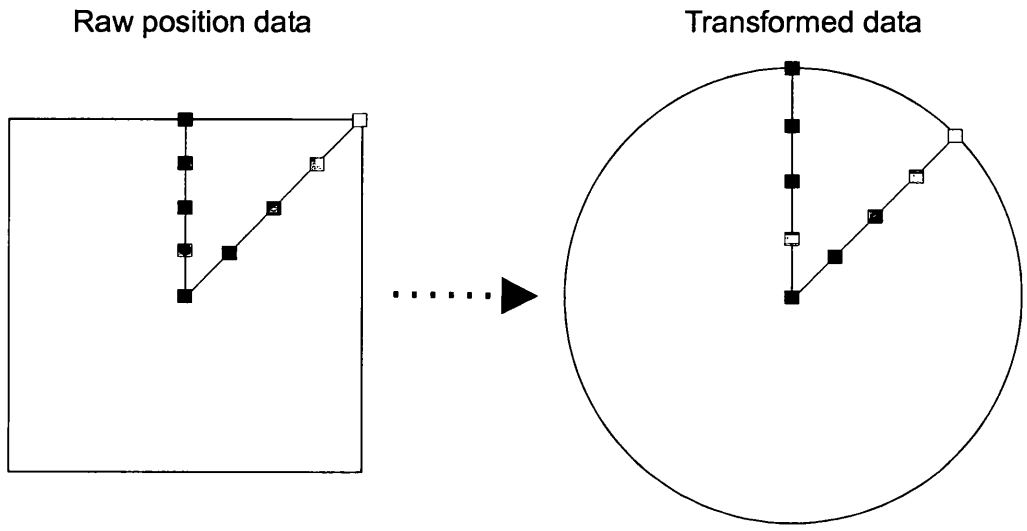


Fig VI.3 Visual guide to the topological transformation used to convert the position data from the square into circular form.

All points in the square were mapped into those of a cylinder occupying the same centre as the square, and the same circumference as the square's perimeter.

The transformation works along radial lines from the centre to the edge of the environment. Each point preserves its radial position as a *proportion of the distance* from the centre to the perimeter along that radius.

In this diagram, pixels of the same colour in the square and circle are topologically equivalent in the transformation.

and was used to measure the "compactness" of the locational fields of TD, PC and HD cells.
A spatial autocorrelogram (ϕ_s) is derived by estimating the similarity in the firing rates in all pairs of bins as a function of the distance between the bins. This procedure is very similar to the one used to construct temporal autocorrelograms described above. We arbitrarily chose 15 bins for the spatial autocorrelograms used in this thesis.

The entropy of the spatial autocorrelogram (ESA) is then computed and its inverse (IESA) used to quantify the strength of the spatial signal in the data.

This measure, contrary to the information measures described before, is not strongly dependent on smoothing or number of bins in the original dataset (Burgess et al, in preparation).

It has been shown that IESA correlates better than the information measures with the experimenters' intuitive feeling of the strength of the spatial signal carried by place, theta, head direction and topodirectional cells (Burgess et al, in preparation).

Comparison of firing patterns in differently shaped environments

Locational firing of TD cells was compared across recording sessions performed in:

1) environments of the same shape (cylinder), to assess the stability of the firing;
2) differently shaped environments (cylinder, square, open platform).

A bin-by-bin correlation analysis was applied to the firing rate maps obtained under these conditions.

Topological transformations

In order to compare firing patterns across the cylinder and the square it is necessary to topologically transform the data. A software was developed by Neil Burgess which transforms the position data obtained from the rats, following a very similar procedure described in Quirk et al (1992) and Sharp (1997). All points in the square were mapped into those of a cylinder with the same centre and circumference. The transformation preserves the fractional distance of each point to the edge of the environment along a radius (see fig VI.3).

Since the perimeter of the wooden square is smaller than the circumference of the wooden cylinder used, the topologically transformed map was expanded to106% of its size. This scaling value was derived experimentally by calculating the ratio between the average radius of the topologically transformed map and the cylinder map across several trials.

In order to compare firing patterns across the cylinder and the open platform, the open platform positional information was reduced to 77% of its size, no further topological transformation was required given that the open platform is circular. The 77% scaling value for the open platform-to-cylinder transformation was calculated as above.

Correlation analysis

A smoothed locational firing rate map was generated for each trial (smoothing level 5, average number of bins 900). Maps from different trials were compared using a bin-by-bin correlation. Bins in which the rate was zero in both maps were discarded, to avoid artifactually inflating the correlation values resulting from many zero-zero bin correlations. The resulting r^2 values served as an index of the similarity of locational firing across the environments used.

An ANOVA was performed with shape comparison as the independent variable with three levels: cylinder-cylinder, cylinder-square, and cylinder-open platform comparisons.

"Distributive Hypothesis" and PxD analysis

The firing rate of topodirectional cells appears to be modulated both by head direction and location. In analysing how these two variables modulate cellular activity, one must take into account bias due to inhomogeneous sampling of places and directions. For example, when a rat's head is at the periphery of a bounded environment, it is virtually impossible for it to simultaneously face the centre of the environment.

This inhomogeneity of behaviour means that firing rate that is solely dependent on spatial location can be misinterpreted as having a directional correlate and vice versa.
One of the few studies which attempted to quantify the problem of the confound created by the influence of two variables (head direction and location) on the firing rate of place cells is by Muller et al (1994). Their approach consisted in calculating the directional firing that one would predict under the null hypothesis that place cell firing is only modulated by location. The predicted rate as a function of direction is:

$$R_{Pred}(\theta) = \Sigma (R_P T_P(\theta)) / \Sigma T_P(\theta) \qquad (1)$$

where R_P is the firing rate in one pixel and $T_P(\theta)$ is the time spent facing head direction θ in that pixel. They then tested whether the observed $R_{Obs}(\theta)$ differed from $R_{Pred}(\theta)$. This was done by computing a "distributive ratio", DR:

$$DR = \Sigma \mid \ln ((1 + R_{Obs}(\theta)) / (1 + R_{Pred}(\theta)) \mid / N \qquad (2)$$

where N is the number of directional bins considered. For a perfect prediction DR is zero. A perfect prediction indicates that the null hypothesis is true, namely place cell firing is only modulated by location and the directional influence on place cell firing is only due to the dishomogeneities of sampling referred to above.

In the case of head direction cells the reverse problem exists: firing solely modulated by directional heading can appear modulated by location. The same

analysis can be applied, by exchanging the place and direction terms in the equations above.

In this thesis the distributive analysis is applied to place, head direction and topodirectional cells. The only modification to the Muller et al method is that predicted firing rates were calculated over all visited bins, rather than only those within the place field. This was done because the background firing rate of topodirectional cells is much higher than that of place cells and therefore cannot be assumed to be zero.

While this analysis can show whether a modulation of firing is artefactual or not, it cannot estimate the relative effect of different variables on firing rate. For this purpose, a new analysis was developed by Neil Burgess (Burgess et al, in preparation).

The expected number of spikes per location and direction bin is modelled as the product of independent functions of the rat's location (p_i) and direction (d_j):

$$E(n_{ij}) = p_i d_j t_{ij} \tag{3}$$

A maximum likelihood approach is then used to choose the values of p_i and d_j that maximise the probability of the observed data. It is assumed that the probability distribution of the observed data follows a Poisson distribution. Thus the likelihood of the data in bin *ij*, given a Poisson model is:

$$p(n_{ij} \mid p_i, d_j, t_{ij}) = (\lambda ij)^{n_{ij}} \exp(-\lambda_{ij})/n_{ij}! \tag{4}$$

Where $\lambda = p_i d_j t_{ij}$. The likelihood of the data, assuming independence over bins is:

$$L = \prod_{ij} p(n_{ij} \mid p_i, d_j, t_{ij}) \tag{5}$$

and the log likelihood is:

$$l = \sum_{ij} \log(p(n_{ij} \mid p_i, d_j)) \tag{6}$$

$$= \sum_{ij} (n_{ij} \log(\lambda_{ij}) - \lambda_{ij} - \log(n_{ij}!)) \tag{7}$$

$$= \sum_{ij} (n_{ij} \log(p_i d_j t_{ij}) - p_i d_j t_i - \log(n_{ij}!) \tag{8}$$

By setting $\partial l / \partial p_i = 0$ and $\partial l / \partial d_j = 0$ we see that the values of p_i and d_i that maximize l (and L) obey:

$$p_i = \frac{< n_{ij} >_j}{< d_j t_{ij} >_j} \tag{9}$$

$$d_i = \frac{< n_{ij} >_i}{< p_i t_{ij} >_i} \tag{10}$$

where $< . >_m$ denotes the average over index m. These equations are then iterated to find p_i and d_j. The values are then scaled to match the total number of spikes recorded and the "corrected" locational maps and directional plots are displayed.

Histology

After completion of the experiments, each rat was killed with an overdose of sodium pentobarbital (Lethobarb, 10mg) and perfused transcardially with saline followed by 4% paraformaldehyde. The brain was extracted and stored in 4% paraformaldehyde, and later sliced coronally into 40 μm thick sections, which were mounted and Cresyl-Violet Nissl-stained to aid visualisation of the electrode track and tip relative to cell bodies.

Results

Chapter VII

Histology

The main topic of this thesis is the study of the firing characteristics of a class of cells, named topodirectional cells. These were recorded from various regions within the hippocampal-parahippocampal formation. Most of the cells were recorded from the subicular cortex, in particular from the dorsal extent of the presubiculum, two from dorsal parasubiculum and one was recorded from the hippocampus proper, just below the stratum pyramidale.

Throughout this thesis cells will be identified by a letter followed by a number. The letter indicates the animal the cell was recorded from, the number the temporal order of recording. Firing characteristics of topodirectional cells (TD) will be compared to those of head direction (HD) and place (PC) cells. The appropriate prefix (TD, HD or PC) will identify cell type. For example: cell TD b4 is the fourth topodirectional (TD) cell recorded from animal b, and cell HD f2 is the second head direction (HD) cell recorded from animal f.

Topodirectional and head direction cells were recorded from a total of 7 and 10 rats, respectively. In three animals (rats a, e and f) both topodirectional and head direction cells were found.

This chapter presents the histological findings for 5 out of the 7 animals from which topodirectional cells were recorded (in two cases the electrode tracks could not be identified: in rat A because the four electrode tracks might have healed during the long time that elapsed between their implant and animal's death; in animal E because 8 tetrodes were implanted and TD cells were recorded from only one of these tetrodes. It proved impossible to conclusively identify the track left in the brain by the relevant tetrode), and a selection of the animals in which only head direction cells were encountered. Where possible a reconstruction of the electrode placement at recording time is given to help identifying the anatomical locations of topodirectional and head direction cells. Fig VII.1 shows a coronal section through the presubiculum, to help the reader to identify the recording sites.

The histological results for the animals from which topodirectional cells were recorded will be presented first.

Rat A

Rat A yielded the majority of topodirectional cells (28 out of 47), and two head direction cells. Four stereotrodes were implanted in the left hemisphere of this

rat, just above the dorsal presubiculum at coordinates: AP –6.7, ML 3.0, DV 1.6 from Bregma, based on the stereotaxic atlas by Paxinos and Watson (1986; this atlas has been used throughout). Unfortunately, it was not possible to identify either of the four expected electrode tracks in the histological sections. This is probably due to the very long time elapsed between electrodes' implant and animal death (more than 4 months), during which healing processes have likely occurred. It has been noted before in monkeys that "tracks made more than a month before perfusion become difficult to find" (Goldberg, 1983).

It is very hard to judge the anatomical source of the topodirectional cells recorded from animal A, given the absence of histological results. Implant coordinates are usually only a crude guide to the actual location of the electrodes in the post-mortem examination. For instance, in our experience, the use of stereotaxic coordinates from the atlas by Paxinos and Watson (1986) generally results in electrode placements more posterior than those intended, in the rat strain used in this study (male Lister hooded). This was found to be true in most of the cases shown in this thesis. It is therefore likely that in the particular case of rat A the actual anterior-posterior placement of the electrodes was somewhat more posterior than the quoted 6.7 mm from Bregma.

It is possible to calculate the theoretical depths (along the dorso-ventral axis) at which both topodirectional and head direction cells were found. These ranged from 2.450 to 2.500 mm ventral to the brain surface. The electrodes were moved ventrally 800 μm before encountering the first two topodirectional cells, left in place for 13 days, and then moved ventrally by a further 50 μm. From then onwards no intentional electrode movement was caused, although it is more than likely that some downward drift of the electrodes occurred during the three extra months while recording took place.

Judging on the basis of the considerations given above, the most likely source of topodirectional signal in this animal were either the white matter just dorsal to the presubiculum or the deep layers of the dorsal presubiculum. It is impossible to tell if the topodirectional and head direction signals recorded in rat A were generated by fibers or cell bodies. The waveform shape analysis presented in next chapter does not unequivocally resolve this question. In this animal head direction and topodirectional cells were never simultaneously recorded but both types of cells were recorded from the same stereotrodes. In particular TD cells were recorded before and after each of the two HD cells, indicating that these two cell types are interspersed.

Rat B

Rat B contributed 5 topodirectional cells over two days (cells b1, b2, b3 during day one and b4-5 next day). A total of 8 independently movable tetrodes were implanted in the left hemisphere of this rat. The tetrodes were arranged in a circular ring, spanning a diameter of 1mm. All topodirectional cells were recorded from one tetrode, whose implant coordinates were: AP 7.65, ML 3.55, DV 3.0. No electrode movement was required to record the five topodirectional cells. It was impossible to unequivocally identify the electrode track made by the relevant tetrode. The first three topodirectional cells were found 12 days after the electrodes were implanted. This

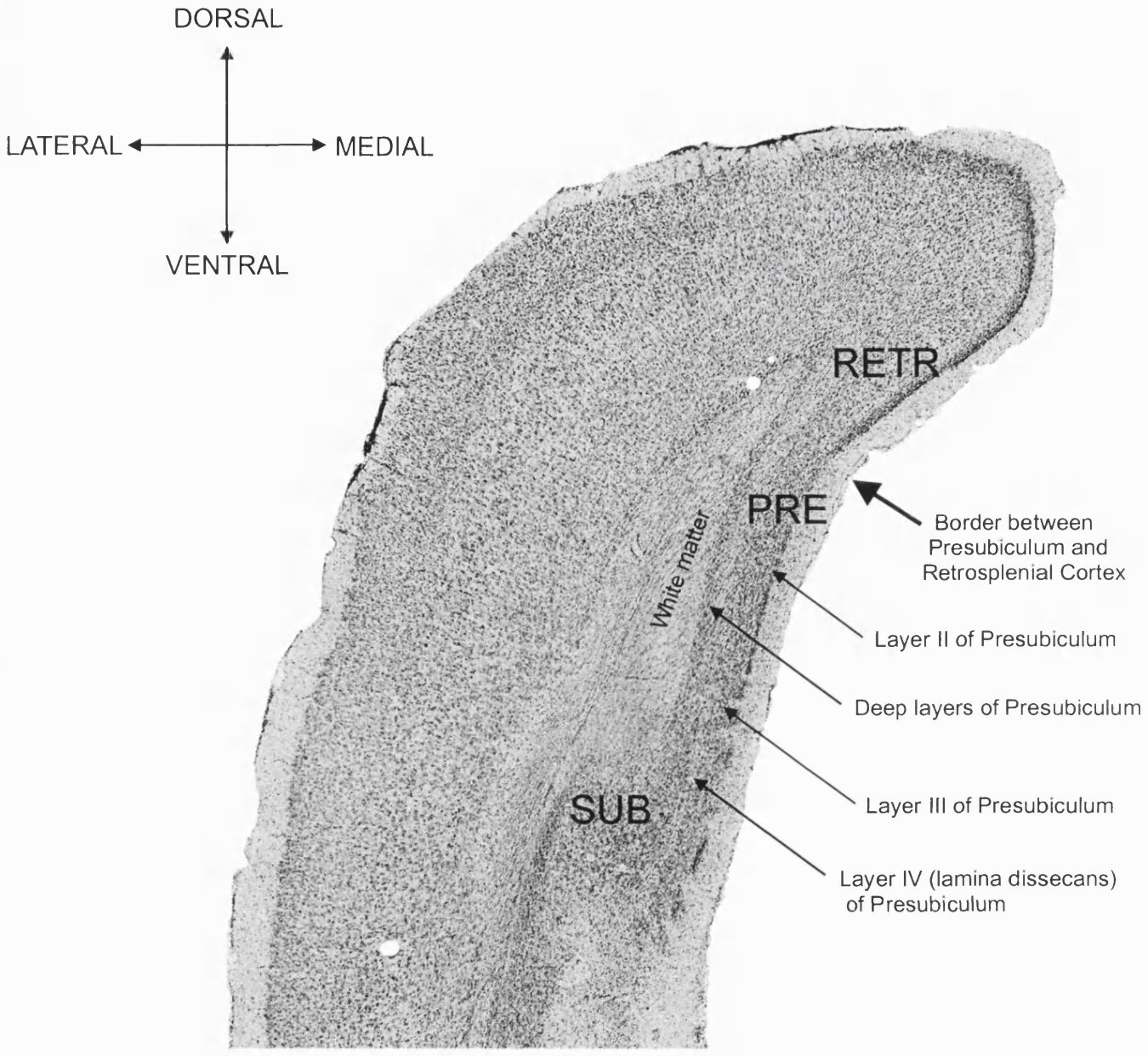


Fig VII.1 Coronal section depicting Presubicular cortex. Section shows dorsalmost two-thirds of left hemispheric cortex at the level of the posterior subiculum. Thick arrow denotes border between presubiculum and retrosplenial cortex. Thinner arrows indicate different layers of the presubiculum.

Abbreviations:
RETR = Retrosplenial cortex
PRE = Presubiculum
SUB = Subiculum

suggests that during such a short time the electrodes could have only drifted ventrally a small amount from the original implant depth (3.0 mm ventral to brain surface).

In conclusion, the most likely source of topodirectional cells in this animal, judged on the basis of the implant coordinates, are the deep layers of dorsal presubiculum. The waveform shapes of the TD cells recorded from this animal are reminiscent of hippocampal pyramidal cells, suggesting that the source of TD signal in this case is more likely to be the somata of principal cells.

Rat C

Only one topodirectional cell was recorded from this animal. Two tetrodes were implanted in the left hemisphere at coordinates: AP –6.7, ML 3.0, DV 1.6. The two tetrodes were staggered by 500 μm and the topodirectional cell was recorded from the shallowest tetrode. Fig VII.2 shows two photomicrographs of a coronal section of this animal's brain where the whole length of one electrode track is visible (the section is roughly equivalent to a level 8.0/8.1 mm posterior to Bregma). This track was made by the two tetrodes, which were closely appositioned. It cuts through the deep and superficial layers of dorsal presubiculum. The topodirectional cell was recorded 50 days before the animal was killed, and during this time, the electrodes were lowered 350 μm. Given that the tip of the longest tetrode is not visible (the tetrode cut through the superficial layers of presubiculum), it is not possible to determine exactly where the tip of the shallowest tetrode is in the fixed brain (at time of death). It is therefore not possible to tell if the topodirectional cell was recorded from either the deep or the superficial layers of presubiculum. It is possible though to discard the idea that the topodirectional signal came from the white matter above the presubiculum.

In summary, cell TD c1 was most likely recorded from the presubiculum.

Rat D

Two topodirectional cells were recorded from this rat. Two microdrives, each carrying two tetrodes were implanted one in the right and one in the left hemisphere. Coordinates were: AP –7.6, ML 3.6, DV 3.0, for the right, and AP –7.7, ML 3.6, DV 3.0, for the left hemisphere.

The first topodirectional cell found (TD d1), was recorded from the left hemisphere. The tetrodes were advanced 40 μm beyond the recording site during the two days which intervened between recording and the animal's death. The tetrodes penetration occurred along an oblique plane, running from dorso-anterior to ventro-caudal positions. Fig VII.3 shows photomicrographs of a series of sections, ordered from posterior to anterior levels. Each section is 40 μm thick. Two electrode tips appear in section B, both in the superficial layers of what could be either para- or presubiculum. The cell was recorded from the more lateral tetrode of the two. If we

consider that what we are seeing in this section are the tips of both tetrodes, we expect the place where the cell was recorded from to be approximately 40 μm more anteriorly than section B. This would correspond to somewhere between sections C and E, allowing for some error. This would mean that the source of the topodirectional signal were the superficial layers of either the pre- or parasubiculum.

The other topodirectional cell (TD d2) recorded in this animal was found in the right hemisphere. The animal was killed just 30 minutes after cell d2 was recorded, therefore the histological results are a very accurate guide to the anatomical location of the cell. Two electrodes tips are visible in the brain section shown in fig VII.4 A and B. The cell was recorded from the more ventrolateral tetrodes, whose tip is at the border between layer I and layer II of parasubiculum.

In summary both topodirectional cells found in rat D were recorded from the superficial layers of the pre/parasubicular cortices.

Rat E

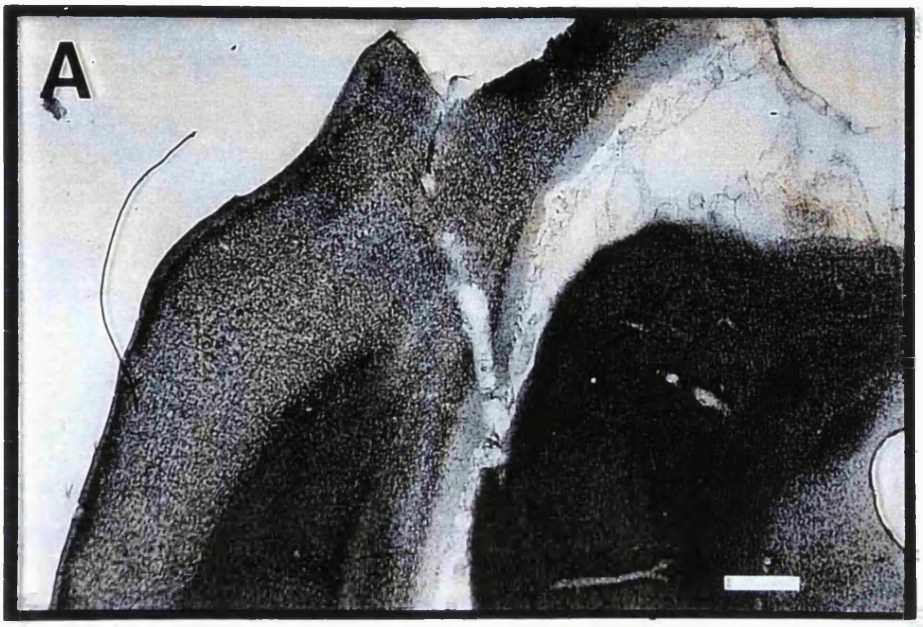
Three topodirectional and seven head direction cells were recorded from rat E. Four tetrodes (cut to the same length) were implanted in the left hemisphere at coordinates: AP –7.6, ML 3.6, DV 2.250. Fig VII.5A shows a photomicrograph of one brain section from this rat's brain, at a level about 7.8 mm posterior to Bregma. The electrode track is quite big, being produced by all four closely appositioned tetrodes. The darkly stained material is blood, testifying to the extensive damage caused by the electrodes. Topodirectional cells were found almost immediately after implant (no movement of the electrodes was required to record TD e1 and e2). Cell TD e2 was simultaneously recorded with a head direction cell (HD e1), but the cells were recorded from separate tetrodes. Three head direction cells (HD e2-4) were simultaneously recorded after the electrodes were moved ventrally of 100 μm, at a supposed depth of 2.350 mm below the brain surface. The electrodes were moved a further 375 μm before the last TD cell was recorded (TD e3; supposed depth 2.725 mm below the brain surface). More HD cells were recorded at a computed depth of 3.200 mm below brain surface (the electrodes were moved a total of 425 μm beyond the last TD cell was recorded). The animal was killed 36 days after the last TD cell was found. This would place the TD cell recording sites in the white mater above the presubiculum for cells TD e1 and e2, and in its the deep layers for cell TD e3. Similarly, some of the head direction signals (HD e1-4) could have come from white matter, while others (HD e5-7) from the deep layers of presubiculum. Topodirectional cells were recorded from two tetrodes and head direction cells from three tetrodes, no cells were recorded from the fourth tetrode. It follows that both topodirectional and head direction cells were recorded from two of the tetrodes. It was never the case that both TD and HD cells were simultaneously recorded from the same tetrode.

These results indicate that in rat E, head direction and topodirectional cells were found in both the white matter overlying the presubiculum and its deep layers.

Fig VII.2 Photomicrographs of coronal sections from animal C showing tracks of tetrodes from which a topodirectional cell was recorded, and indicating this cell was recorded from the presubiculum.

A, B) A depicts section showing single track made by two tetrodes very closely appositioned, B shows higher magnification detail of same section. Section is equivalent to a level about 8.0/8.1 mm posterior to bregma in atlas of Paxinos and Watson (1986). There was virtually no horizontal separation between the two tetrodes (this is why only one track is visible): the tip of one was 500 μm above the other. A topodirectional cell was recorded from the shallower, shorter tetrode of the two (it is not possible to visualize the tip of this tetrode, given that the longest tetrode cut through presubicular superficial layers). The animal was killed 50 days after the topodirectional cell was recorded. During this 50-day period, the drive was lowered 350 μm beyond the recording site.

Scale bar in A = 625 μm, scale bar in B = 310 μm.


A

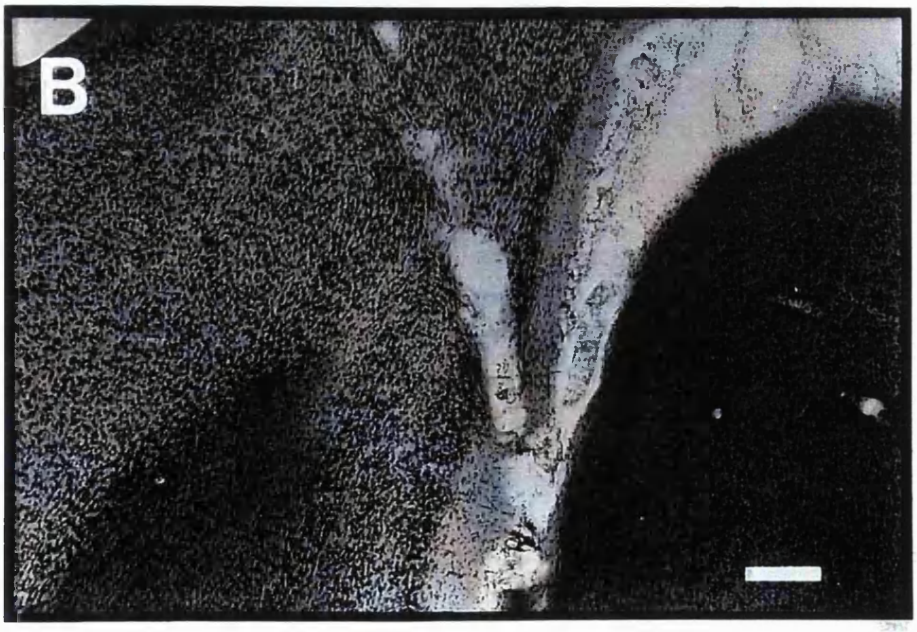

B

Fig VII.3 Photomicrograph series of successive, adjacent coronal sections from the left hemisphere of animal D showing tracks of tetrodes from which a topodirectional cell was recorded, and suggesting the cell was recorded from superficial layers of the parasubiculum or presubiculum.

A – F) The series runs from posterior (A) to anterior (F). The ventralmost portions of two tetrode tracks are depicted. The tetrodes ran obliquely; their entry was dorsal-anterior, going ventro-caudal. Accordingly, most of the dorsal extent of the track is not visible on these sections. Sections were cut 40 μm thick.

A topodirectional cell was recorded from the more lateral tetrode of the two (see white arrow in D). The animal was killed two days after the topodirectional cell was recorded. During this two-day period, the drive was lowered 40 μm beyond the recording site. The sections are taken from a part of the brain where, for a fixed mediolateral-dorsoventral point, the transition from parasubiculum posteriorly to presubiculum anteriorly is quite rapid. Given that the drive was lowered 40 μm beyond the recording site, there is some degree of doubt as to whether the cell was recorded from the presubiculum or parasubiculum. However, even allowing for a wider range than 40 μm of movement from the tip of the lateral tetrode track (visible from B-F, not clearly visible in A), the tetrode would clearly be in the superficial layers. The "best guess" for the recording site is presubiculum layer III.

Scale bar in A = 650 μm, and also applies to B-F.

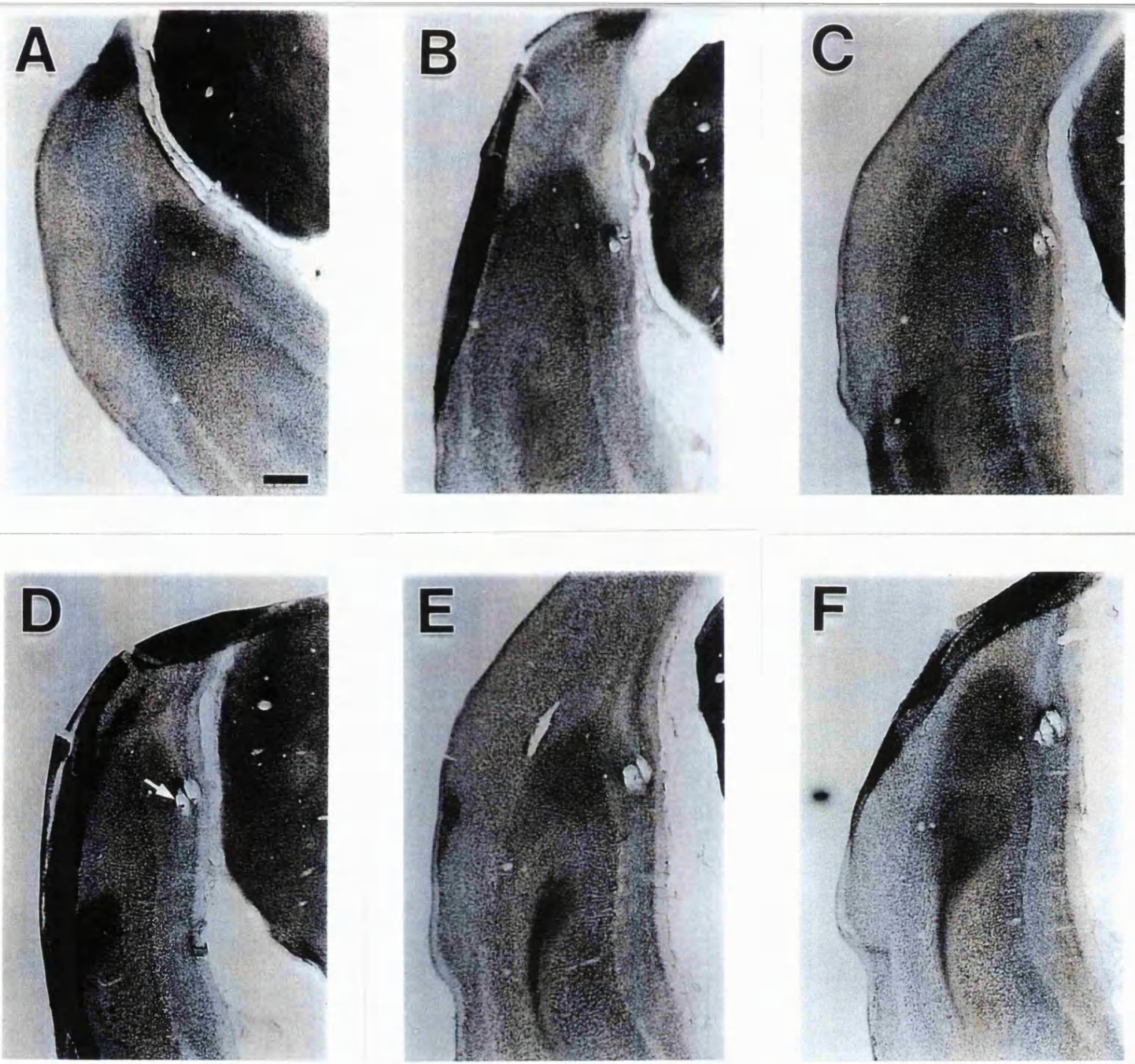
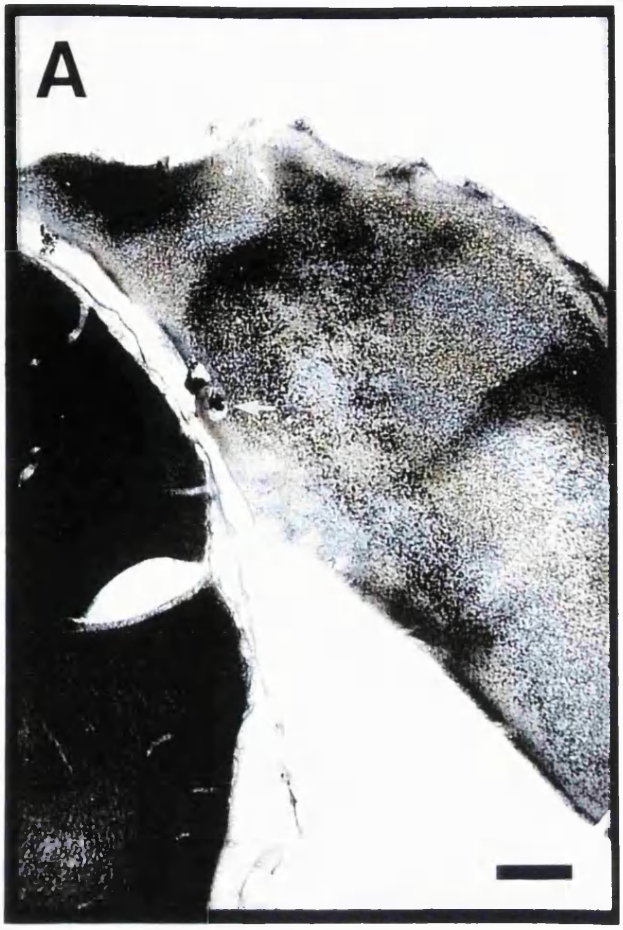

A
B
C
D
E
F

Fig VII.4 Photomicrographs of coronal sections from right hemisphere of animal D showing tracks of tetrodes from which topodirectional cells were recorded, and suggesting that these cells were recorded from superficial layers of the parasubiculum.

A, B) A depicts section showing ventralmost portions of two tetrode tracks, B shows higher magnification detail of same section. (The tetrodes were not positioned vertically, and thus most of the dorsal extent of the track is not visible on this section. The entry was dorsal-anterior, going ventro-caudal.) Section is equivalent to a level about 8.9 mm posterior to bregma in atlas of Paxinos and Watson (1986). Topodirectional cells were recorded from the more ventrolateral tetrode of the two, whose tip apposes layer I and the external portion of the external lamina of the parasubiculum (layer II; see white arrow in A). The animal was killed 30 minutes after the last topodirectional cell was recorded, with no alteration to drive depth.

C) Anterior adjacent section to that in A & B, showing ventrolateral tetrode track tip apposing layer I and the external portion of the external lamina of the parasubiculum (layer II).

Scale bar in A = 625 μm, scale bar in B = 313 μm, and applies also to C.


A


B


C

Fig VII.5 Photomicrographs of coronal sections from animal E (A) and G (B) showing tracks of electrodes from which topodirectional cells were recorded.

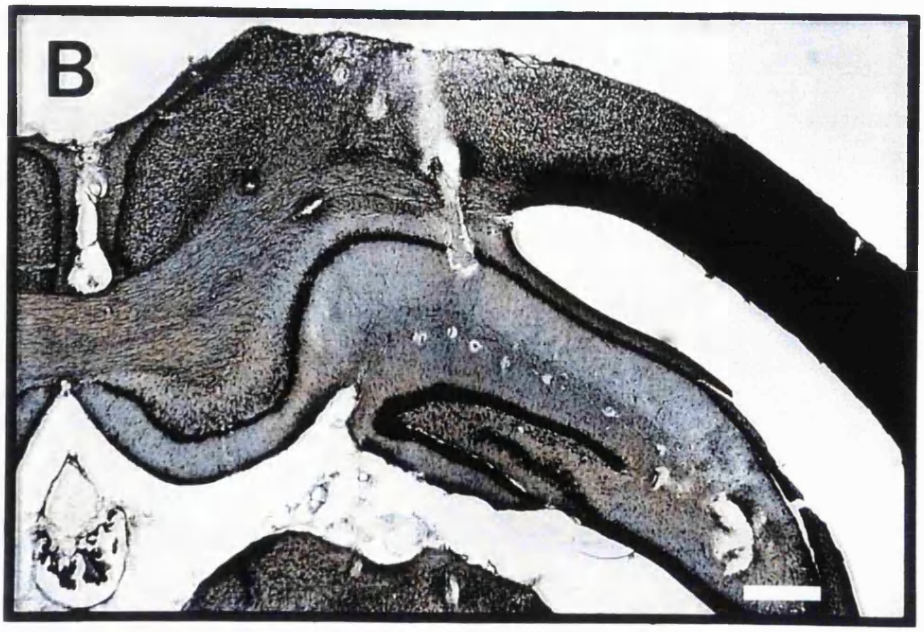
A) Section from animal E showing large track region made by 4 tetrodes closely appositioned (only one track, derived from all four tetrodes, is visible). Section is equivalent to a level about 7.8 mm posterior to bregma in the atlas of Paxinos and Watson (1986). Tips are located around layers IV/V/VI of the presubiculum. Topodirectional cells were recorded from two of the four tetrodes. The animal was killed 36 days after the last TD cell was recorded. During this 36-day period, the drive was lowered 1 mm beyond the last TD-cell recording site. Accordingly, it may be speculated that TD cells were recorded from white matter above the presubiculum.

Scale bar = 675 μm.

B) Section from animal G showing tetrode track having passed below dorsal hippocampal CA1 layer. The animal was killed one day after the TD cell was recorded. Note that the topodirectional signal recorded was reversed in polarity, and may have been axonal.

Scale bar (white) = 625 μm.


A


B

Rat F

In total, 7 topodirectional and 7 head direction cells were recorded from this animal. Four tetrodes were implanted in the left hemisphere at coordinates: AP –7.6, ML 3.2, DV 1.8. The first topodirectional cell (TD f1) was recorded by the tetrode whose track is the most medial of the two shown in fig VII.6. This cell was recorded after the electrodes were moved 400 μm beyond implant site, at a theoretical depth of 2.200 mm below the brain surface. It was recorded 47 days before the animal's death, during which time the electrodes were moved a total of 200 μm beyond the cell's recording site.

Without further electrodes' movement more TD cells (TD f2-6) and all the HD (HD f1-7) cells were recorded from the two tetrodes whose tracks are shown in two successive brain sections in fig VII.6 A-D. These cells were recorded over a period of 20 days. In two instances both topodirectional and head direction cells were recorded simultaneously and from the same tetrode. In the first case one TD (TD f3) and two HD (HD f1-2) cells were simultaneously recorded, and next day one TD (TD f4) and one HD (HD f3) were recorded together. At the end of this 20 day period the electrodes were moved downward 100 μm during 12 days and the last TD cell was encountered (TD f7). After this, the electrodes were moved a further 100 μm and the rat killed 15 days after this last TD cell was recorded.

This brief description indicates that cell TD f1 (fig VII.6 E-F), was probably recorded from the deep layers of presubiculum (at least 200 μm above the electrode tip of the most medial track in fig VII.6 E-F). Cells TD f2-6 and HD f1-7 were recorded from the tetrodes whose tracks are shown in fig VII.6 A-D, at least 200 μm above the position of the tips in the fixed brain. This would probably place the cells in the deep layers of presubiculum. Cell TD f7 was recorded from the most medial tetrode in fig VII.6 A-D and a least 100 μm above its tip, possibly in presubicular layer III.

In summary, the histological results presented above suggest that TD and HD cells were mainly recorded from the deep layers of presubiculum. TD and HD cells were simultaneously recorded, from the same tetrode, in two separate occasions, confirming that these two cell populations are not anatomically segregated in the rat's brain.

Rat G

Only one TD cell was recorded from rat G. Two tetrodes were implanted in the right hemisphere of this animal aiming for the hippocampus proper at coordinates: AP –3.8, ML 2.4, DV 1.5. The electrodes were lowered 250 μm and several place cells were recorded over a period of 9 days. The electrodes were then moved a further 400 μm and one topodirectional signal of inverted polarity was encountered. One place cell was simultaneously recorded with the topodirectional unit. The animal was killed one day after the TD unit was found and the electrode track made by the tetrode from which the TD signal was recorded is shown in fig VII.5 B. The electrode tip is

below the hippocampal pyramidal layer. It is possible that the source of topodirectional signal in this animal was an axon, given that the recorded waveform was reversed in polarity.

Rats from which only HD cells were recorded

Fig VII.7, presents the histological findings for 4 out of the 10 animals from which head direction cells were recorded (three more animals from which head direction cells were recorded were rat A, E and F described in detail above). In general, head direction cells were recorded from the deep layers of presubiculum, with the exception of rat J where they were possibly recorded from both superficial and deep layers of this structure (fig VII.7 E-F). In all these animals no topodirectional cells were ever recorded.

Summary and discussion

The histological findings described above suggest that topodirectional cells were most likely found in: 1) the white matter overlying the dorsal presubiculum, 2) the deep and superficial layers of dorsal presubiculum, 3) the superficial layers of parasubiculum. One topodirectional cell was also recorded from a location just below the pyramidal layer in the hippocampus proper. In that case the signal was probably generated by an axon (see Table 0).

More than once, it was possible to record topodirectional and head direction cells simultaneously, sometimes even from the same tetrode, indicating that these cell types are anatomically very close in the brain. Very similarly to what has already been reported of head direction and place cells, no topographical arrangement of topodirectional cells was apparent. Simultaneously recorded cells show widely different directional and locational preferences, and cells recorded at different levels along an electrode track never showed progressive changes in either their directional or locational fields.

It was often possible to record from several topodirectional cells at once, suggesting that these cells are organised in "islands" or clusters within the brain structures listed above. This was also found to be true of head direction cells.

We are aware of only two cells with similar characteristics to those of topodirectional cells. They were recorded by Sharp (1996) in the presubiculum, and showed all three hallmarks of topodirectional firing: locational, directional and theta modulation of their firing rate. Three more cells that might have shown very similar firing characteristics to those of TD cells were recorded in the parasubiculum by Taube (1995c). These cells showed a clear locational and directional modulation of their firing rate. Given that theta modulation of their firing was not assessed, we cannot discard the possibility that these cells presented all three TD hallmarks. Even though it is slightly surprising that topodirectional cells have not been described before, a reason for this might be the relative low amplitudes of their signals, as will be shown in next chapter (chapter VIII, waveform analysis). Usually recordings in the pre- and parasubiculum have been made using single wires, while in this thesis

Table 0	
cell	**anatomical location**
tda1-28	not known
tdb1-5	not known
tdc1	presubiculum
tdd1	sup layers of either pre or parasubiculum
tdd2	border between layers I and II of parasubiculum
td e1-2	white matter above presubiculum
td e3	deep layers of presubiculum
tdf1-6	deep layers of presubiculum
tdf7	layer III of presubiculum
tdg1	below CA1 pyramidal layer

Fig VII.6 Photomicrographs of coronal sections from animal F showing tracks of electrodes from which topodirectional cells and head direction cells were recorded, and suggesting that these cells were recorded from layers III-VI of the presubiculum.

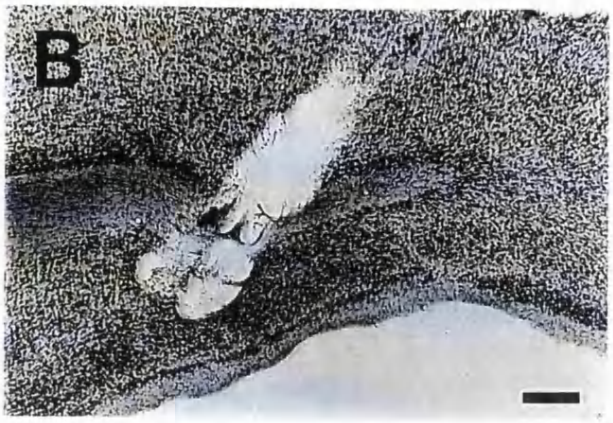
Three sections are depicted, in pairs of photos: those on the right show higher-magnification details from the views seen on the left. Sections are arranged from posterior (top of page: A, B) to anterior (bottom of page: E, F). Animal F was implanted with four tetrodes. The tracks of the two tetrodes from which most cells were recorded are shown in A-D. The section in C & D is adjacent to that in A & B, which is equivalent to a level 7.7/7.85 mm posterior to bregma in the Paxinos and Watson (1986) atlas. Sections were 40 μm thick.

A, B, C, D) Topodirectional cells were recorded over a 32-day period from the two tetrodes whose placements are shown in A-D. Head direction cells were also recorded from these tetrodes at the beginning and middle of this 32-day period. The tip of the more medial of the two tetrodes is in presubicular layer III. The tip of the more lateral tetrode is around presubicular layer III/IV/V. By the end of the 32-day period, the drive had been lowered by a total of 100 μm from the first recording site. The animal was killed 11 days after the end of the 32-day period, the drive having been lowered another 100 μm, but with no further cells recorded. The at-death placement of the lateral tetrode, and the fact that topodirectional cells were recorded from the lateral tetrode before the drive had been lowered by 200 μm overall, probably indicate that topodirectional cells can be recorded from the deep layers. In general, reconstructions from both the lateral and medial tetrodes suggest that topodirectional and head direction cells were recorded in the region of layers III to VI.

E, F) The section in E & F is about 280 μm anterior in the fixed brain to that in A & B. The tip of the tetrode track is in presubicular layer III. One topodirectional cell was recorded from this tetrode. The recording was done 4 days before the start of the 32-day recording period referred to above (47 days before the death-date). No head direction cells were recorded from this tetrode.

Scale bars in A, C & E = 680 μm, scale bars in B, D & F = 340 μm.


A


B


C


D


E


F

Fig VII.7 Photomicrographs of coronal sections from animals L (A, B), K (C), H (D), and J (E, F) respectively, showing tracks of electrodes, placed in the presubiculum, from which head direction cells were recorded.

A, B) A depicts section from animal L showing two tetrode tracks, B shows higher magnification detail of same section. Section is equivalent to a level about 7.6 mm posterior to bregma in atlas of Paxinos and Watson (1986). Tips of both tracks are located in presubicular layer III. Head direction cells were recorded from both tetrodes. The animal was killed 10 days after the last HD cell was recorded. During this 10-day period, the drive was lowered 350 μm beyond the last HD-cell recording site.

C) Section from animal K showing two tetrode tracks. Section is equivalent to a level about 6.7 mm posterior to bregma in atlas of Paxinos and Watson (1986). Tip of more ventral track is in presubicular layer III, tip of more dorsal track is around presubicular layer III/IV. Head direction cells were recorded from both tetrodes. The animal was killed 6 days after the last HD cell was recorded. During this 6-day period, the drive was lowered 225 μm beyond the last HD-cell recording site.

D) Section from animal H showing two stereotrode tracks. Section is equivalent to a level about 7.6 mm posterior to bregma in atlas of Paxinos and Watson (1986). Head direction cells were recorded from the more medial of the two stereotrodes (see white arrow). The tip of the more medial track is in deep presubicular layers (V/superficial VI). The animal was killed 1 day after the last HD cell was recorded, with no alteration to drive depth.

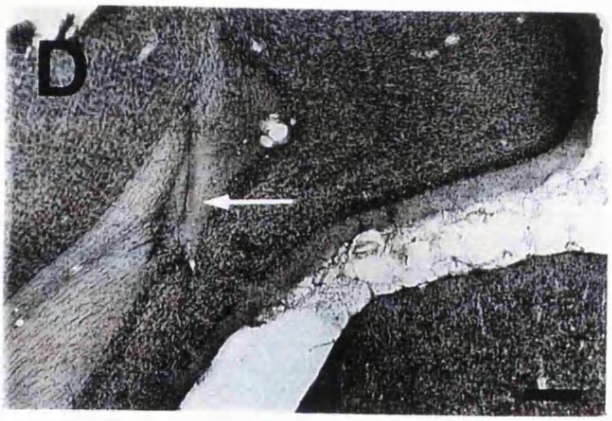
E, F) E depicts section from animal J showing single track made by two tetrodes closely appositioned, F shows higher magnification detail of same section. Section is equivalent to a level about 7.6 mm posterior to bregma in atlas of Paxinos and Watson (1986). Tip of track is around presubicular layers II/III. Head direction cells were recorded from both of the tetrodes over a range of more than 600 μm along the tetrode tracks. The animal was killed 17 days after the last HD cell was recorded. During this 17-day period, the drive was lowered 350 μm beyond the last HD-cell recording site.

Scale bars in A, D, and E = 680 μm, scale bars in B, C, and F = 340 μm.


A


B


C


D


E


F

stereotrodes and more often tetrodes were used, making it easier to isolate and discriminate small amplitude signals, particularly when more than one signal was present (this was quite often the case, given that both TD and HD cells tend to occur in clusters).

Chapter VIII

Waveform analysis

This chapter presents the results of the waveform shape analysis for topodirectional and head direction cells. In particular, spike amplitude and duration will be considered, as well as the general shape of spikes generated by the two cell types. This analysis is intended to help in identifying the anatomical source of the topodirectional signal in the brain: are topodirectional units most likely interneurons, axons or principal cells? Therefore, the results presented here will be discussed with reference to those of the previous chapter.

Methods

In total 46 topodirectional (TD) and 62 head direction (HD) cells were included in this analysis. TD cells were recorded from 8 hemispheres in 7 rats (rat a = 28 cells, b = 5, c = 1, d = 2, e = 3, f = 6, g = 1), while HD cells were recorded from 10 rats (rat a = 2 cells, e = 7, f = 7, h = 5, i = 1, j = 17, k = 12, l = 6, m = 1, n = 4).
The data used in this study were collected from 8 minute recording trials in the standard cylindrical environment (see chapter VI, p. 65). When more than one trial was recorded, the particular trial chosen was that in which the cell fired the greatest number of spikes.

Results

For each cell, the averaged waveform of all the spikes recorded during the selected trial is presented in fig VIII.1. As a comparison, the averaged waveforms from the 62 head direction cells (HD) are shown in fig VIII.2. Nomenclature is as defined in the previous chapter.

In most cases TD cells' waveforms are tri-phasic, being characterised by the succession of a negative-positive-negative phase (but see waveforms for cells TDb1-5, TDd1-2, TDf2 and TDg1; in all pictures negative is up).
HD cells' waveforms show a different pattern, with most of them displaying similar shapes to those of hippocampal pyramidal cells, characterised by a negative phase, an ascending positive phase and a long, smooth afterhyperpolarisation which brings the membrane potential back to resting values.
Note that on some occasions the signals recorded had an inverted polarity (e.g. TDa14, TDa28, TDf5, HDa2, HDe3, HDj2).

The spike height (peak-to-peak voltage) and the spike width (time between the first and second peak) were calculated for both TD and HD cells (see fig VIII.3;

Fig VIII.1 Waveforms of the entire topodirectional cell sample (46 cells).

Calibration: For each cell, the scale on the y axis denotes μV, the horizontal line through "0" denoting the resting or baseline potential. The length of the entire x axis is equivalent to 1millisecond.

The waveforms of most topodirectional cells in the sample are triphasic, with a shorter spike width than those of head direction cells (see figure VIII.1). The spike heights are also smaller than those of head direction cells; it seems likely that this contributes to undersampling of the topodirectional cell population. Although more information matching cell type to waveform shape is required, most topodirectional cell waveforms are suggestive of signals from interneurons. There are however, significant exceptions: see cells from animal B(TD b1+b5), D (TDd1+2), and F (TD f2).

Overall the evidence suggests that topodirectional cells are from a heterogenous population comprised mainly of interneurons.

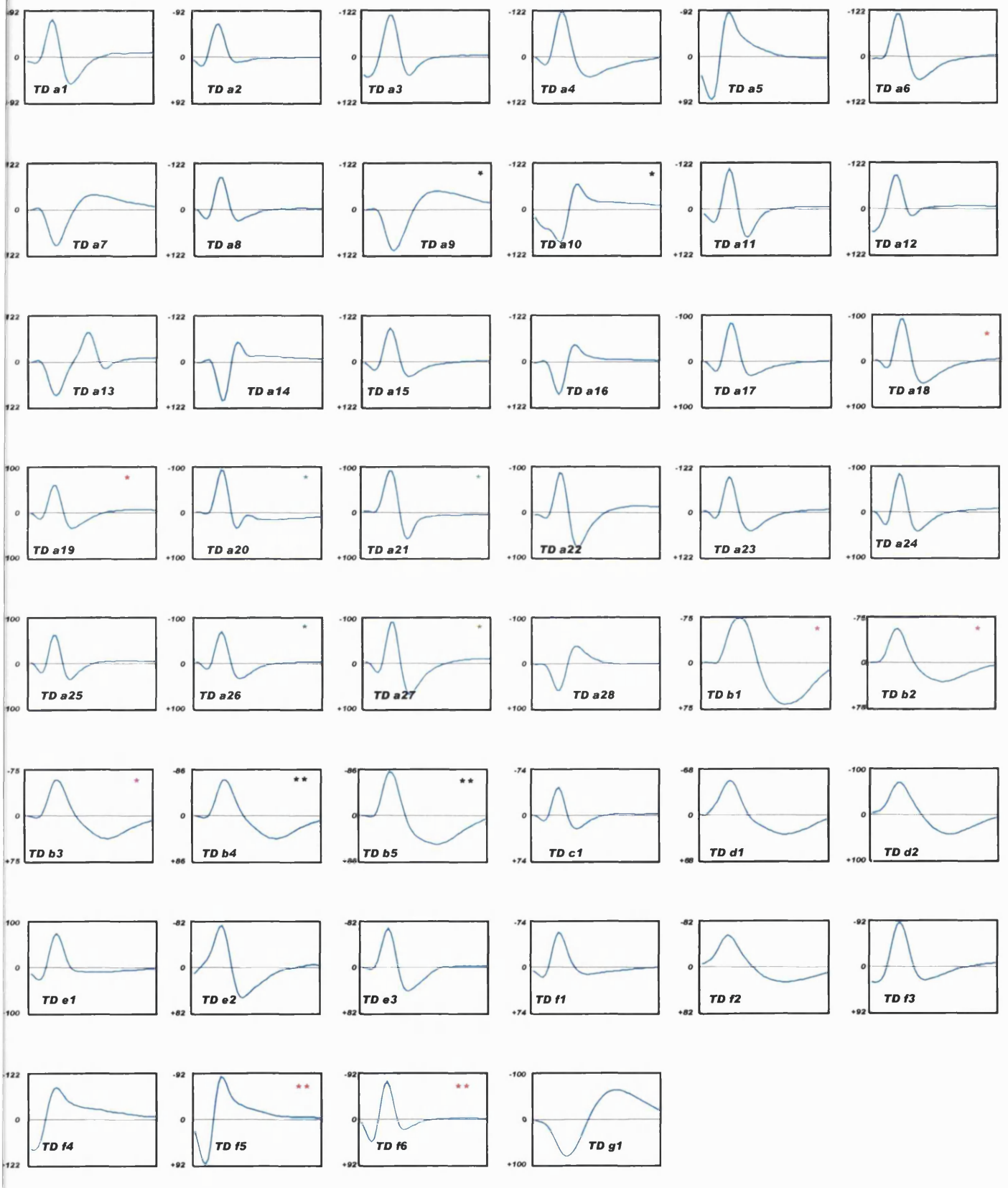
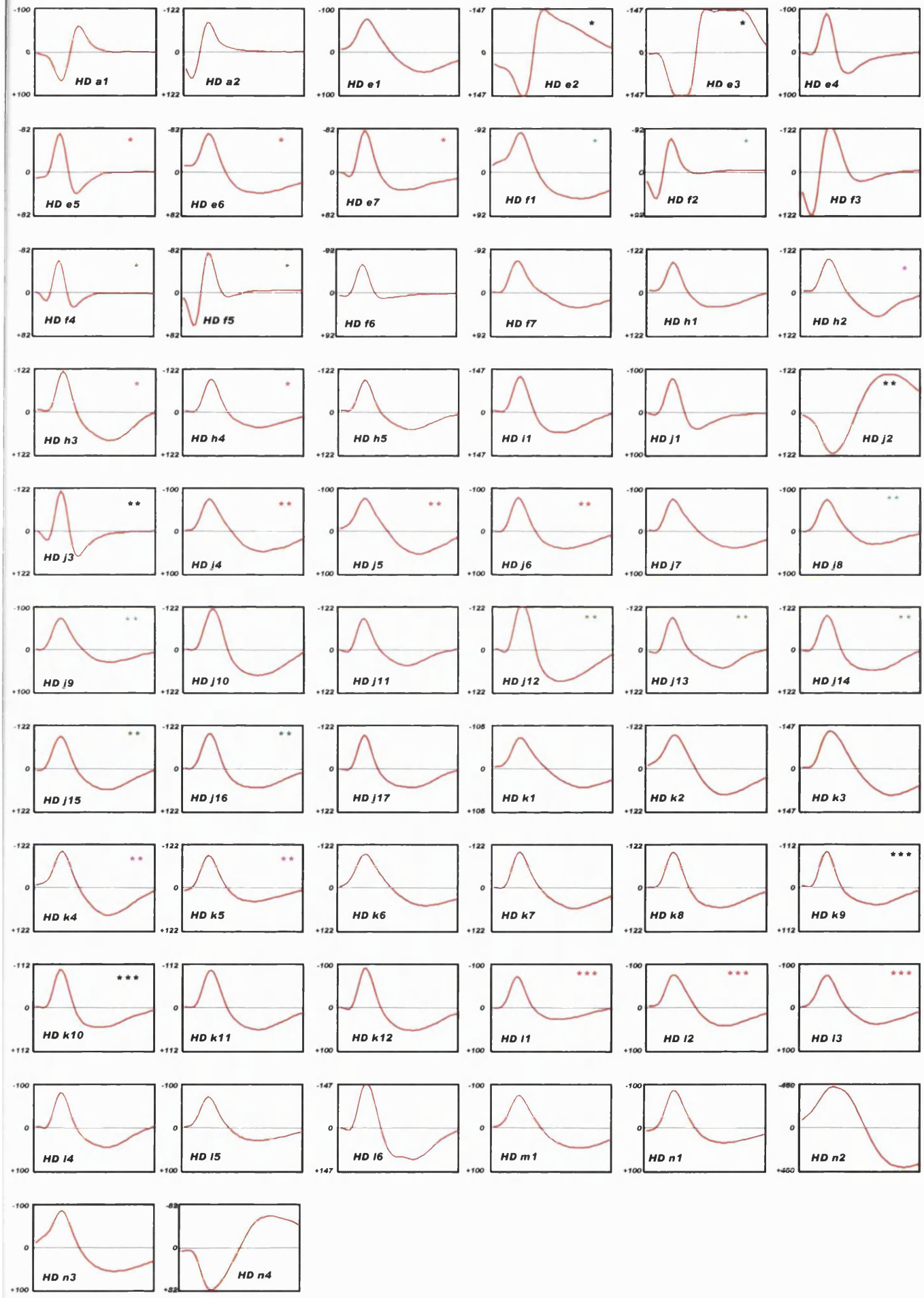

TD a1
TD a2
TD a3
TD a4
TD a5
TD a6
TD a7
TD a8
TD a9
TD a10
TD a11
TD a12
TD a13
TD a14
TD a15
TD a16
TD a17
TD a18
TD a19
TD a20
TD a21
TD a22
TD a23
TD a24
TD a25
TD a26
TD a27
TD a28
TD b1
TD b2
TD b3
TD b4
TD b5
TD c1
TD d1
TD d2
TD e1
TD e2
TD e3
TD f1
TD f2
TD f3
TD f4
TD f5
TD f6
TD g1

Fig VIII.2 Waveforms of the entire head direction cell sample (62 cells).

Calibration: For each cell, the scale on the y axis denotes μV, the horizontal line through "0" denoting the resting or baseline potential. The length of the entire x-axis is equivalent to 1 millisecond.

In this and subsequent figures, the letter after HD refers to the animal the cell was recorded from. The number after this letter denotes the temporal order in which the cell was encountered, except that shared asterisks denote cells recorded simultaneously from the same stereo/tetrode. Thus, for example: i) cell HD e2 and HD e3 were recorded simultaneously; ii) Cell HD f7 was recorded after cell HD f6, from the same animal.

Note that most HD cell waveforms are suggestive of signals from large principal cells (pyramidal or stellate) rather than interneurons or axons.


HD a1
HD a2
HD e1
HD e2
HD e3
HD e4
HD e5
HD e6
HD e7
HD f1
HD f2
HD f3
HD f4
HD f5
HD f6
HD f7
HD h1
HD h2
HD h3
HD h4
HD h5
HD i1
HD j1
HD j2
HD j3
HD j4
HD j5
HD j6
HD j7
HD j8
HD j9
HD j10
HD j11
HD j12
HD j13
HD j14
HD j15
HD j16
HD j17
HD k1
HD k2
HD k3
HD k4
HD k5
HD k6
HD k7
HD k8
HD k9
HD k10
HD k11
HD k12
HD l1
HD l2
HD l3
HD l4
HD l5
HD l6
HD m1
HD n1
HD n2
HD n3
HD n4

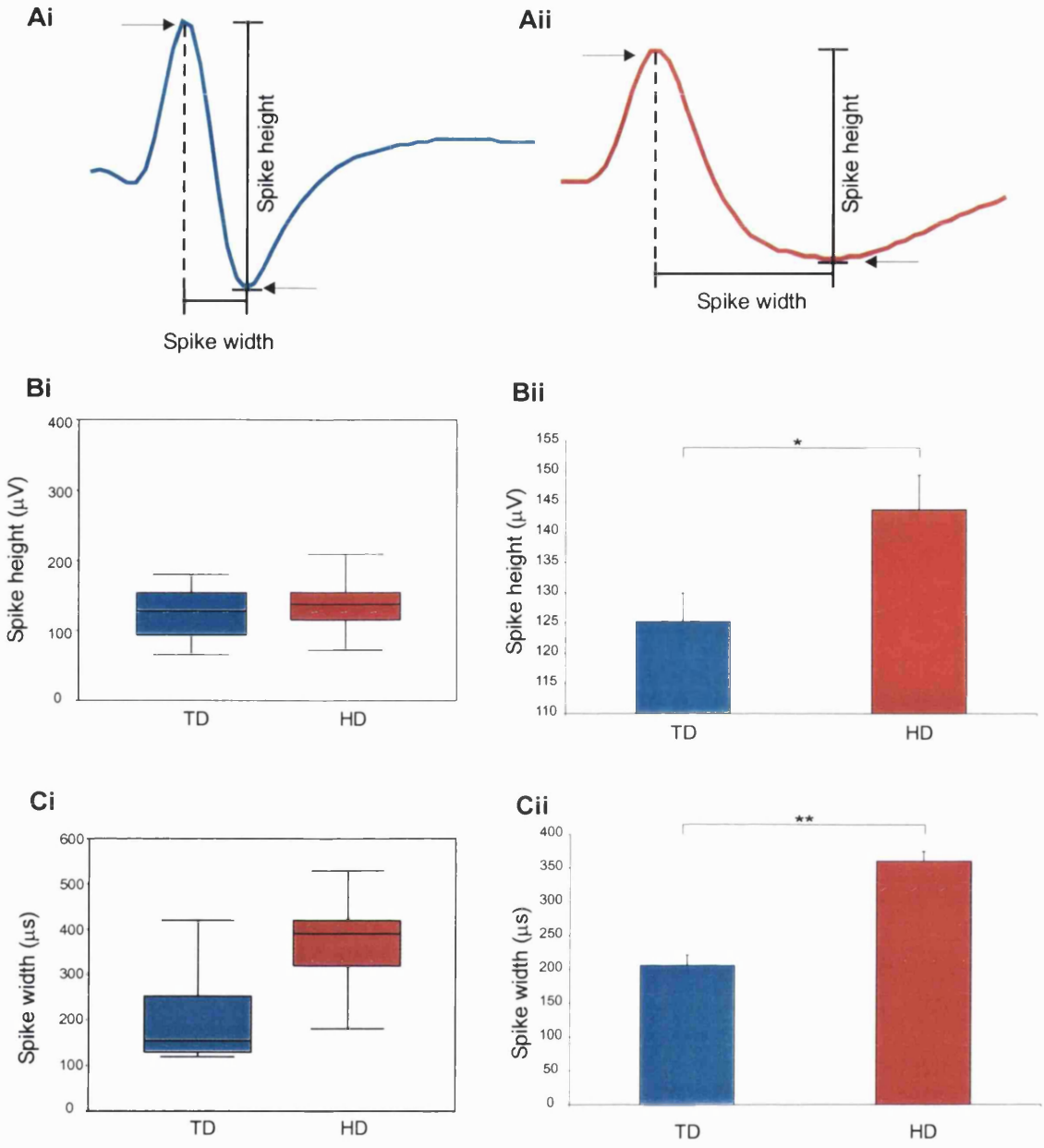


Fig VIII.3 Analysis of waveforms of topodirectional (blue) and head direction (red) cell samples.

A) Visual illustration of what is defined as 'spike height' and 'spike width' for a representative topodirectional cell (Ai) and head direction cell (Aii). Arrows point to peak and trough of spike.

B) Box plot (Bi) and mean and standard error plot (Bii) of spike height (μV) in topodirectional and head direction cell samples.

C) Box plot (Ci) and mean and standard error plot (Cii) of spike width (μs) in topodirectional and head direction cell samples.

It can be seen that on average the spike heights of the sampled topodirectional cells are smaller, and their spikes widths shorter, than those of the head direction cells. These differences likely reflect differences in the respective cell populations.

bandpass filtering between 500 Hz and 7 kHz). Fig VIII.3 B and C show box plots and mean and standard error plots respectively for spike height and width. The box plot gives a visual representation of the distribution of the data. The box represents the interquartile range, which contains 50% of the values, the whiskers are lines that extend from the box to the highest and lowest values excluding outliers. A line across the box indicates the median. In the mean and standard error plot the height of the bar represents the value of the mean and the line extending from it the standard error of the mean. The average spike height for TD and HD cells was 125.24 (± 4.7) μV and 143.66 (± 5.7) μV respectively. A two-tailed t-test revealed that the mean spike height of TD cells is significantly different from that of HD cells ($df = 105$, $t = -2.4$, $p<0.025$).

The average spike width was 205.87 (±15.3) μs for TD cells and 360.49 (±14.3) μs for HD cells. Again, there is a significant difference between these values ($df = 105$, $t = -7.3$, $p < 0.001$).
Thus, TD cells have spikes of lower amplitude and shorter duration than those of HD cells.

Discussion

The waveform analysis presented is not conclusive with regards to the origin of the TD signal. The source of topodirectional firing could be fibers or cell bodies of either interneurons or principal cells. It is very hard to distinguish between these possibilities on the basis of the waveform of the extracellularly recorded spikes, as very little is known of how waveform shape correlates to neuronal type.
The only clear relation found is that between spike amplitude and cell size: there is a positive correlation between these two variables, ie larger cells generate higher amplitude extracellular signals (Humphrey and Schmidt, 1990). In light of this, the fact that topodirectional spikes are of relatively low amplitude (lower than those of head direction cells), indicates that they are probably generated by small neurons. The low amplitude of topodirectional signal might also explain why this class of cells had not been reported before, as anticipated in the previous chapter.

As regards to waveform shape, very few studies have attempted relating it to neuronal type. It has been shown that hippocampal interneurons generally show short duration spikes, with tri-phasic waveforms, while pyramidal neurons have a longer duration and are characterised by the slow afterhyperpolarisation, as seen in the majority of HD waveforms described above (Ranck, 1973; Csicsvari; 1999). We do not know if this is applicable to pre- and parasubicular interneurons. The only study that described the electrophysiological properties of morphologically distinct cells in pre- and parasubiculum is one by Funahashi and Stewart (1997a) described in chapter II. They recorded intracellularly from *in vitro* preparations and reported that pyramidal and stellate cells in pre- and parasubiculum had indistinguishable membrane properties. They suggested that they probably had not recorded from interneurons.

We have shown that TD spikes have shorter duration than HD spikes and that while the majority of the TD units recorded had triphasic waveforms, some of them are biphasic, with a pyramidal-like waveform shape (TDb1-5, TDd1-2, TDf2 and

TDg1). Conversely, a few HD units recorded had a triphasic waveform, as found in Taube et al (1990a). Thus, the short duration spikes and the tri-phasic shape of the majority of topodirectional cells both indicate that they might be small pre- and parasubicular interneurons (we cannot speculate on the cytological identity of TD cells, given that we were recording extracellularly). On the other hand, HD cells, which have mainly higher amplitude, pyramidal-like, longer duration spikes, are most likely presubicular principal cells.

No simple relationship can be found between waveform shape and anatomical location (as judged by histological analysis), for topodirectional cells. For instance, in animals A and F pyramidal spikes (TD a7, a9 and f2) were recorded both before and after tri-phasic spikes, indicating that there is no clear anatomical demarcation between these two spike types. One interesting finding is that all topodirectional cells recorded from animal B and D have a pyramidal-like waveform. It was not possible to obtain unequivocal histological localisation for the cells recorded from animal B, while the two cells from animal D were recorded from the superficial layers of the pre/parasubicular cortices.

Arguably, in the case of head direction cells a pattern can be found whereby cells recorded earlier (and therefore at deeper levels, see fig. VII.1) present triphasic and often inverted signals, and those recorded later have pyramidal-like waveforms.

It is possible that a few topodirectional and head direction signals recorded were generated by fibers. In particular, the histological data presented in the previous chapter suggest that at least in the case of animal E this was the case.
On the other hand, the fact that for almost all the TD units recorded, a signal could be detected on more than one wire of the same stereo/tetrode (see fig VIII.4 for two examples), makes it highly unlikely that in general, the signal sources were fibers.

In conclusion, both HD and TD signals appear to be generated by a heterogeneous population of presubicular and parasubicular (for TD cells) neurons. We hypothesise that the majority of TD cells are probably interneurons, while the vast majority of HD cells are probably principal cells.

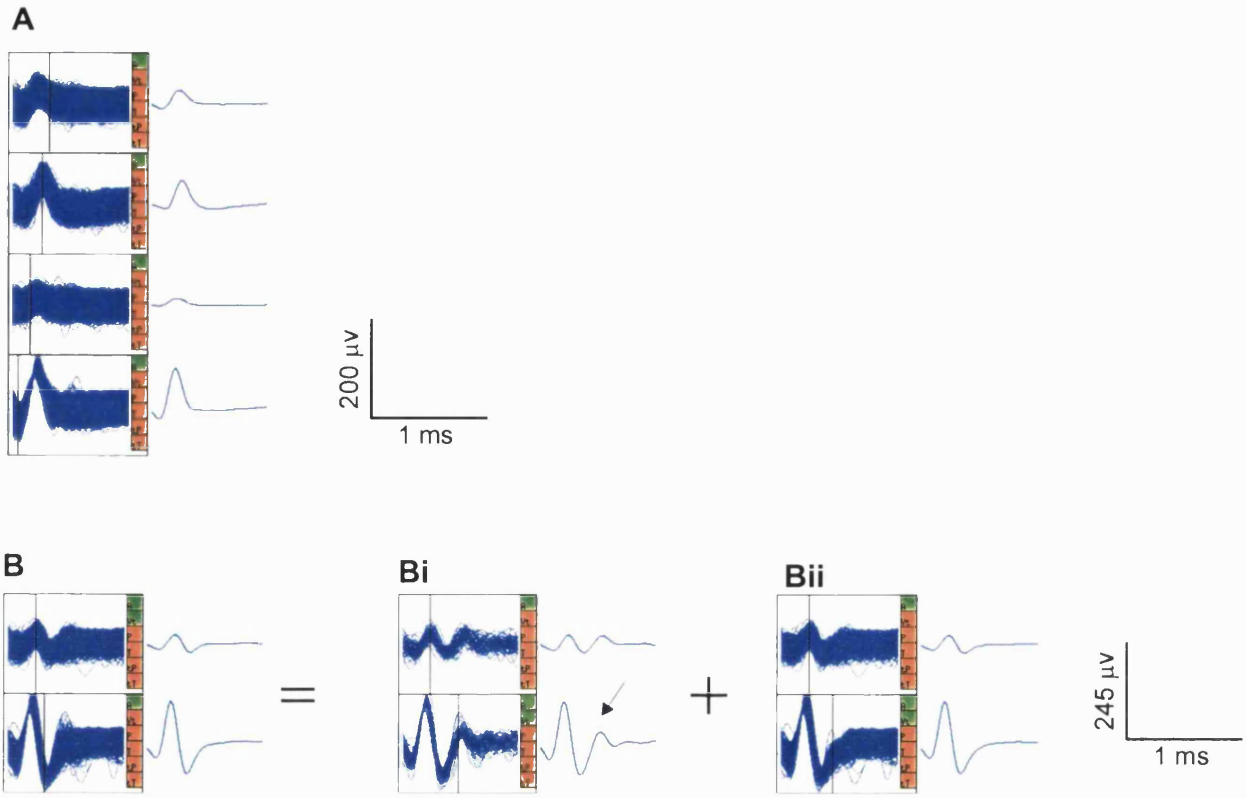


Fig VIII.4 Further features of topodirectional cell waveforms. For each channel, all the spikes are shown on the left of the orange bar, and the averaged waveform is shown to the right of the orange bar.

A) This TD cell was recorded from a tetrode. Note that significant spike heights are obtained on three of the four channels. This makes it unlikely that the signal source is a fiber.

B) This TD cell, from a different animal, was recorded from a stereotrode. All the spikes recorded in the trial are shown in B, left of the equals sign. The spikes can be separated into two types (right of equals sign): Bi, with a second negative component, a small spike about 300 µs after the main spike (see arrow), and Bii) the dominant spike shape without the second component.

This second negative component is reminiscent of waveforms recorded from interneurons of the hippocampus (Csicsvari et al, 1999). It is not clear why only a subset of the spikes shows the second component.

Chapter IX

General Characteristics of Topodirectional Cells

The firing rate of topodirectional cells (TD) is a function of both location and head direction. Fig IX.1 and 2 show two examples of topodirectional cells. Cell TD f3 fires whenever the animal is in the central-north region of the cylinder and is facing south-west (see central map in B for locational field and polar plot in A for directional preference). Also shown are 8 locational firing rate maps each depicting cell firing restricted to a specific 45° range of head directions. For example, the top map shows the cell's locational firing pattern when the animal is facing north, the next map to the right (clockwise) when facing north-east, etc. It is clear from these maps that firing is almost completely restricted in the central region of the cylinder and is strongly modulated by head direction. The proportion of spikes fired by the cell when the rat is facing south-west (the cell's preferred direction), is 10 times higher than that obtained when the animal is facing in the opposite direction. From fig IX.1 it is apparent that this effect cannot be attributed to lack of sampling of the region encompassing the locational field of the cell when the rat is facing north-east.

TD g1 firing is even more strongly modulated by head direction (fig IX.2). When the animal faces in the cell's preferred direction (south) the cell fires 50 times more spikes than when the animal faces in the opposite direction. Firing of topodirectional cells is generally strongly modulated by head direction, similarly to that seen for cell TD g1.

Fig IX.3 shows locational and directional firing rate maps for all the TD cells recorded in a cylinder (N=46). For example, cell TDa7 fires maximally when the rat is in the south-west region of the cylindrical enclosure, pointing his head south, while cell TDf5 has a crescent shaped place field hugging the north-eastern portion of the circle and a east-south-eastern directional field. Cell TDf5 fires maximally when the rat is in the north east portion of the cylinder and is pointing his head to the east.

The firing properties of topodirectional cells will be compared to those of place and head direction cells. Shown in figs IX.4 and 5 are 12 examples of place (PC) and 12 of head direction (HD) cells, chosen from the populations used in the analysis. Cells were selected on the basis of their locational and directional information content. For each spatial information variable (location and direction), two place cells were selected with information values lying at the 25^{th}, median, and 75^{th} percentile of the respective distributions (see fig IX.4). The same procedure was used to select the 12 head direction cells shown in fig IX.5.

It would appear that some place cells show strong directional components to their firing (see cell PC o5 and p5). Similarly, some head direction cells show locationally restricted firing patterns (see cell HD j4 and j14). These results are artefactual, due to uneven sampling of the spatial variables in question (location and head direction), as will be explained in the next chapter.

This chapter provides an overview of the general firing characteristics of TD cells, comparing them to those of head-direction and place cells recorded under very similar conditions.

Methods

Cell Samples

The properties of 46 TD cells were compared with those of 46 HD and 46 PC cells.

TD cells were recorded from 7 rats (rat a = 28 cells, b = 5, c = 1, d = 2, e = 3, f = 6, g = 1).

HD cells were recorded from 10 rats (rat a = 1 cell, e = 7, f = 6, h = 5, i = 1, j = 11, k = 8, l = 5, m = 1, n = 1). HD cells were randomly selected from the total population sample of 62 cells used in the Waveform Analysis chapter.
Note that in some animals both TD and HD cells were recorded (rats a, e and f). Occasionally TD and HD cells were simultaneously recorded.

The place cells included in this chapter were recorded by Colin Lever and Tom Wills, from CA1, in 4 rats (o = 15, p = 24, q = 6, r = 1).

The data used in this chapter was collected from 8 minute recording trials in the standard cylindrical environment (see chapter VI, p.66). The particular trial chosen was that in which the cell fired the greatest number of spikes.

The locational firing rate maps presented and used for statistical analysis contain an average of 900 bins, while the directional plots contain 36 bins (each bin is 10°). Smoothing level for locational maps is set at 5 (see Methods section). No smoothing is applied to directional maps.

Statistical analysis

Several measures of spatial firing were computed across the three cell types. These were: overall firing rate, locational and directional selectivity, place field size and directional firing range. Overall mean firing rate was computed by dividing the total number of spikes the cell fired by recording time and is expressed in Hz. Locational and directional selectivity were calculated as the ratio between the locational (directional) peak firing rate and the overall mean firing rate for that cell. Two spatial information measures were considered: one developed by Skaggs (1993), the other a modification of the former by Burgess (Burgess et al, in preparation). The spatial information measures were computed for both the locational and the directional fields.

The inverse of the spatial entropy function (IESA, see chapter VI, p.70) was computed for the locational fields of PC, TD and HD cells, as a measure of the

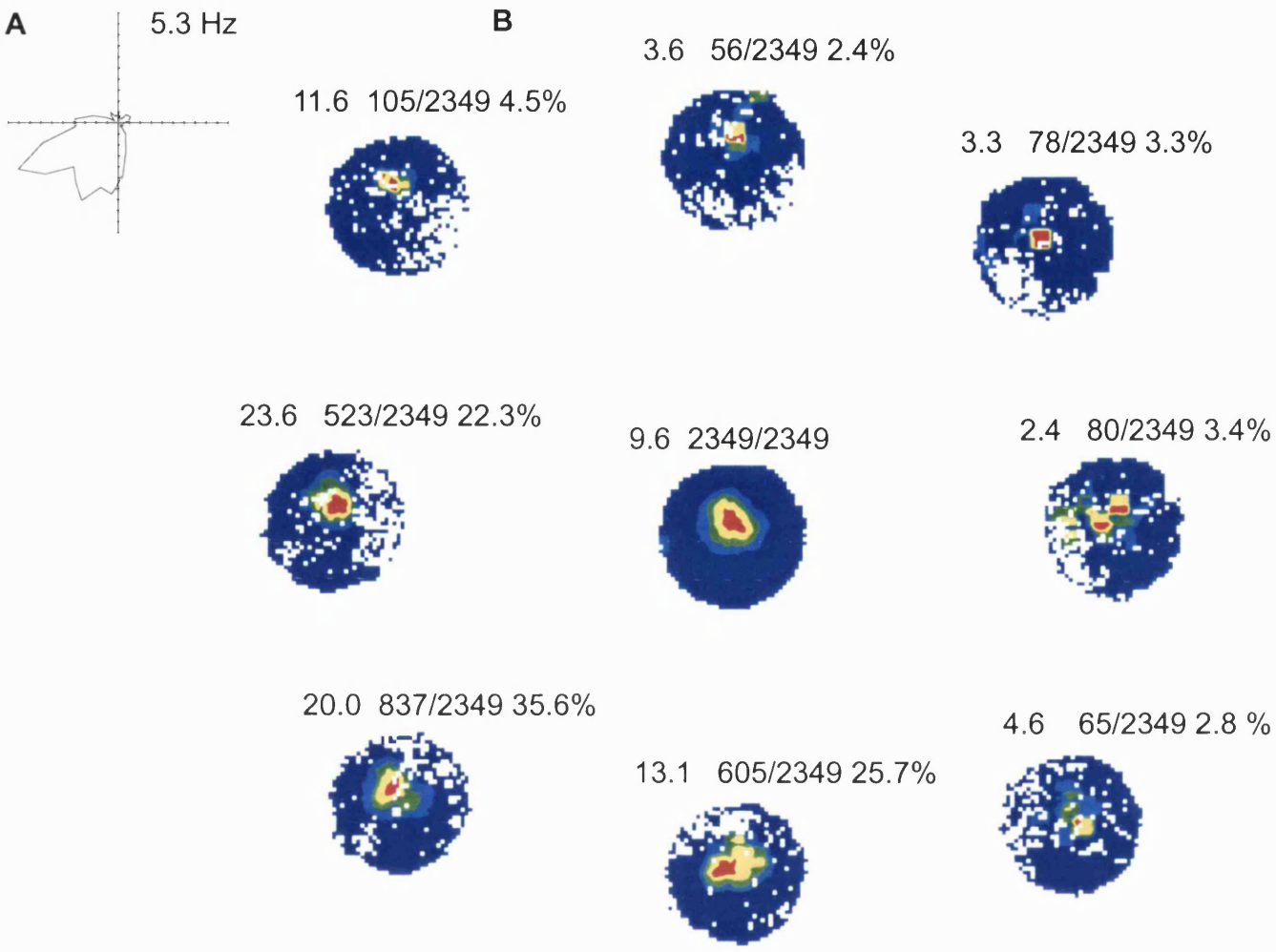


Fig IX.1 Similar position of locational field, but differential directional responses within this field, in a topodirectional cell (TD f3).

A) Directional polar plot showing cell fires maximally when the rat faces south- west.

B) Central firing rate map shows the place field averaged over all directions. Surrounding rate maps show locational firing for specific head directions only (in 45 degree bins.) First number refers to peak rate in field (Hz), second gives number of spikes fired in that direction out of total (= 2349 spikes). This is also expressed as a percentage. Thus, e.g. top right map shows locational firing in the cell's central field with a peak of 3.3 Hz, when the rat faced the north-east direction, and fired 78 spikes (3.3% of the total).

It can be seen that the cell always fires in the central region of the cylinder, and that its firing is modulated by head direction. The cell fires at a much higher rate in its locational field when the rat faces south-west and west.

This cell is more strongly modulated by location than the the average topodirectional cell.

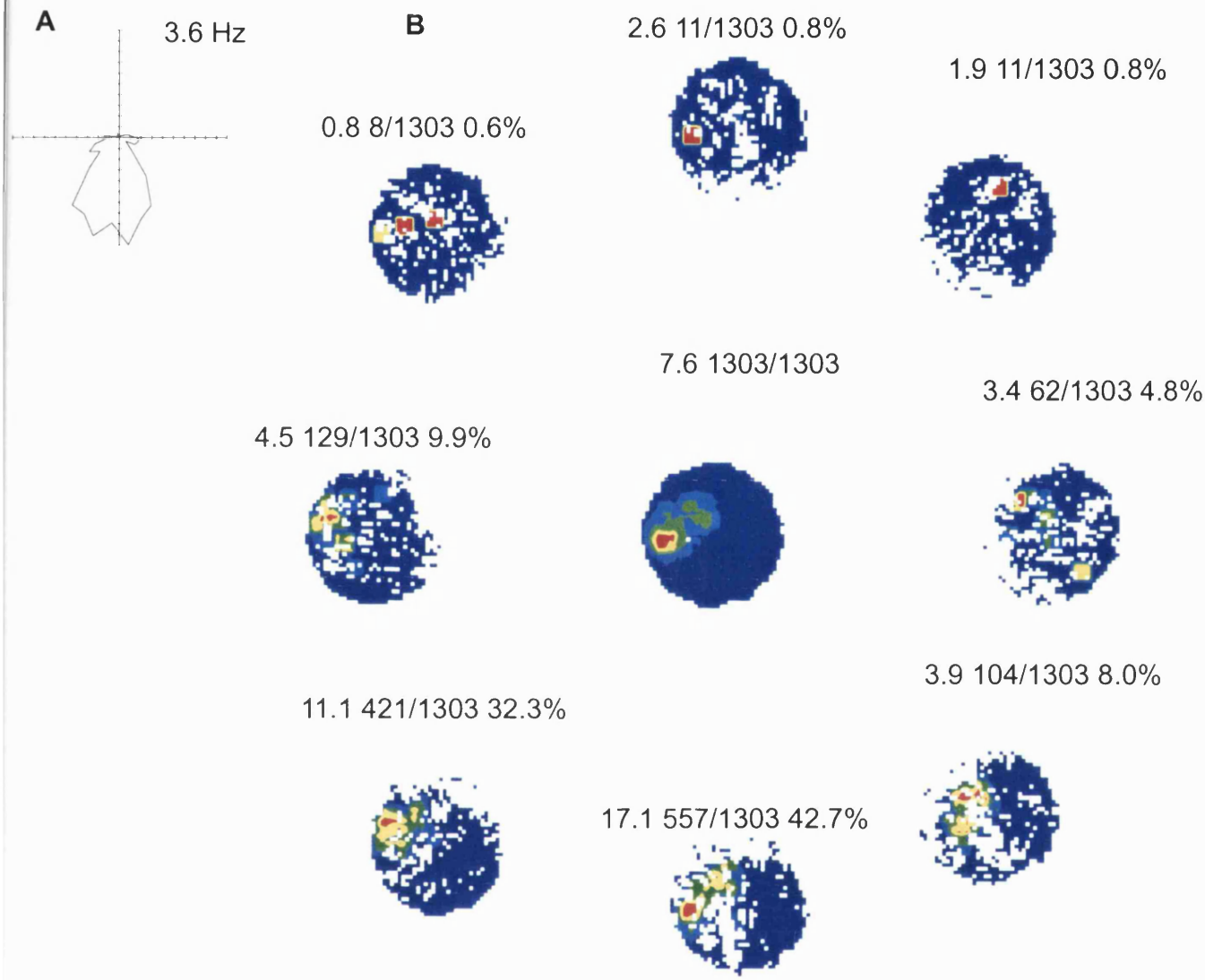


Fig IX.2 Similar position of locational field, but differential directional responses within this field, in a topodirectional cell (TD g1).

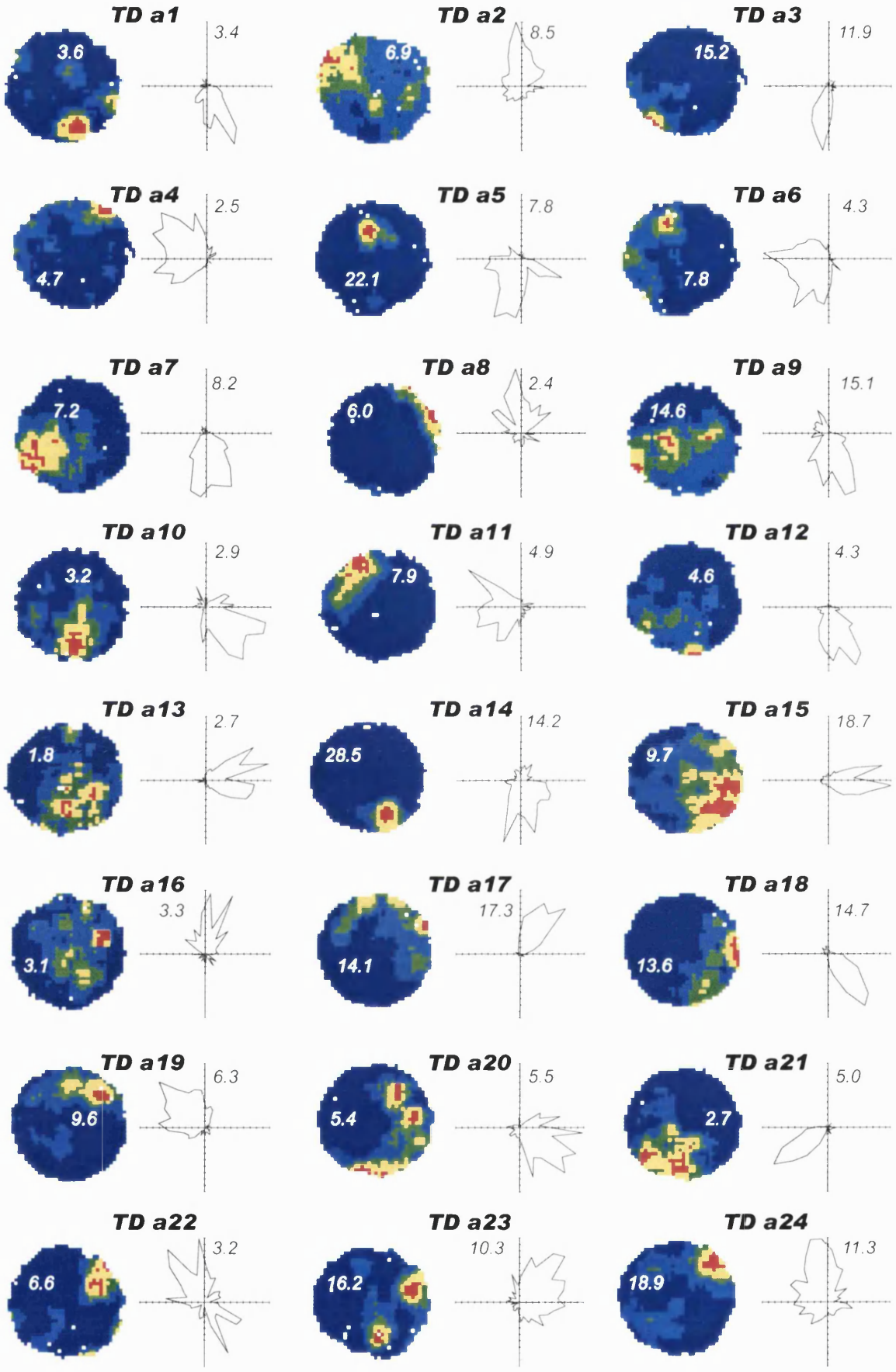
A) Directional polar plot showing cell fires maximally when the rat faces south.

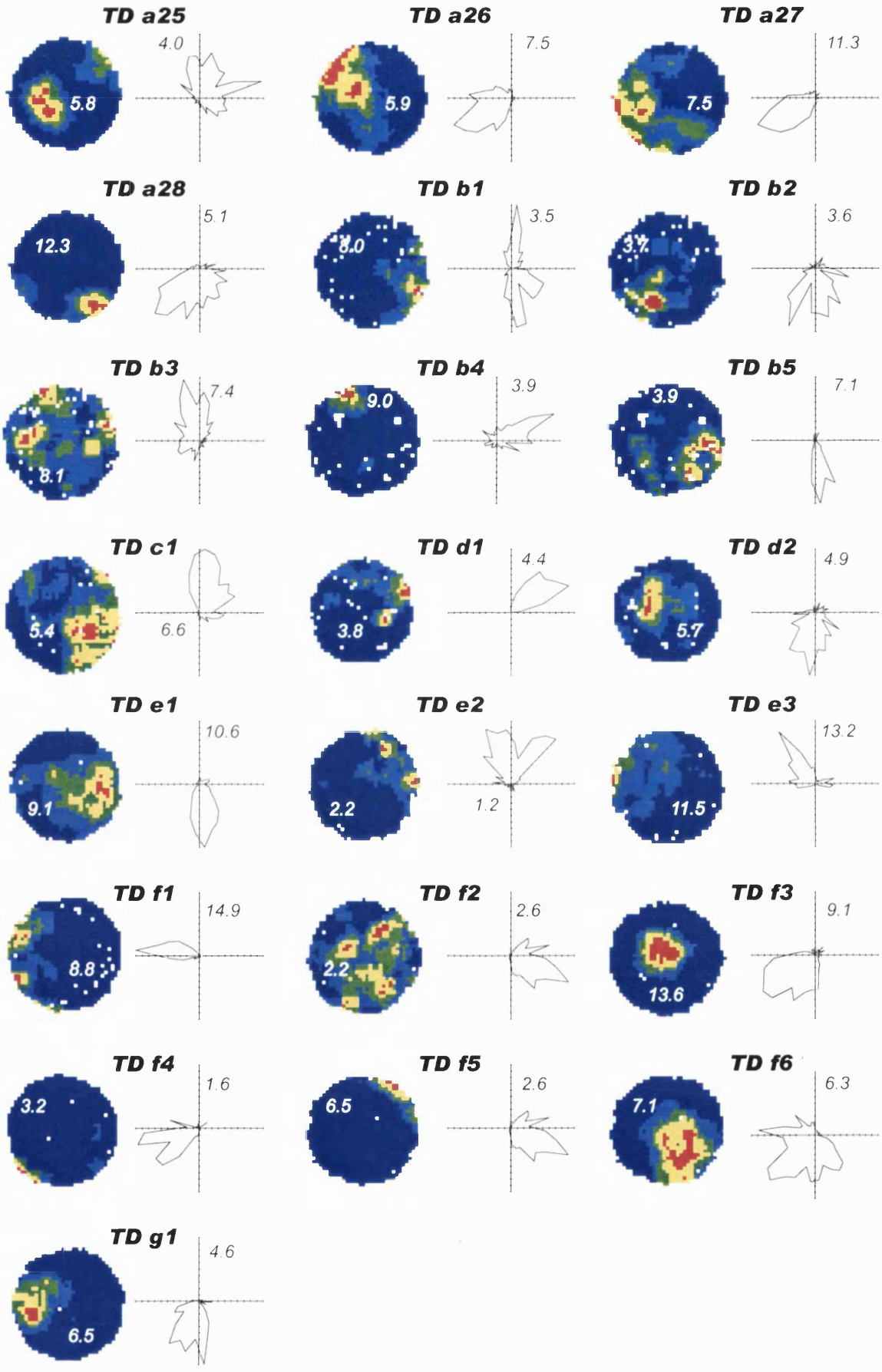
B) Central firing rate map shows the place field averaged over all directions. Surrounding rate maps show locational firing for specific head directions only (in 45 degree bins.) Conventions are as in Figure IX.1.

This cell is more strongly modulated by head direction than that shown in fig IX.1. Firing occurs always in the central-west region of the cylinder, and it is maximal when the rat faces south.

Fig IX.3 Firing rate maps and directional polar plots for the entire sample of topodirectional cells in the canonical cylinder trial.

For each cell, the locational firing rate map (left), and the directional polar plot (right) are shown. The number in white in the rate map, and that at the top of the polar plot, denote the Locational and Directional peak firing rate respectively, in Hz.


TD a1
3.6
3.4
TD a2
6.9
8.5
TD a3
15.2
11.9
TD a4
4.7
2.5
TD a5
22.1
7.8
TD a6
7.8
4.3
TD a7
7.2
8.2
TD a8
6.0
2.4
TD a9
14.6
15.1
TD a10
3.2
2.9
TD a11
7.9
4.9
TD a12
4.6
4.3
TD a13
1.8
2.7
TD a14
28.5
14.2
TD a15
9.7
18.7
TD a16
3.1
3.3
TD a17
14.1
17.3
TD a18
13.6
14.7
TD a19
9.6
6.3
TD a20
5.4
5.5
TD a21
2.7
5.0
TD a22
6.6
3.2
TD a23
16.2
10.3
TD a24
18.9
11.3


TD a25
4.0
5.8
TD a26
7.5
5.9
TD a27
11.3
7.5
TD a28
5.1
12.3
TD b1
3.5
8.0
TD b2
3.6
3.7
TD b3
7.4
8.1
TD b4
3.9
9.0
TD b5
7.1
3.9
TD c1
5.4
6.6
TD d1
4.4
3.8
TD d2
4.9
5.7
TD e1
10.6
9.1
TD e2
2.2
1.2
TD e3
13.2
11.5
TD f1
14.9
8.8
TD f2
2.6
2.2
TD f3
9.1
13.6
TD f4
3.2
1.6
TD f5
6.5
2.6
TD f6
7.1
6.3
TD g1
4.6
6.5

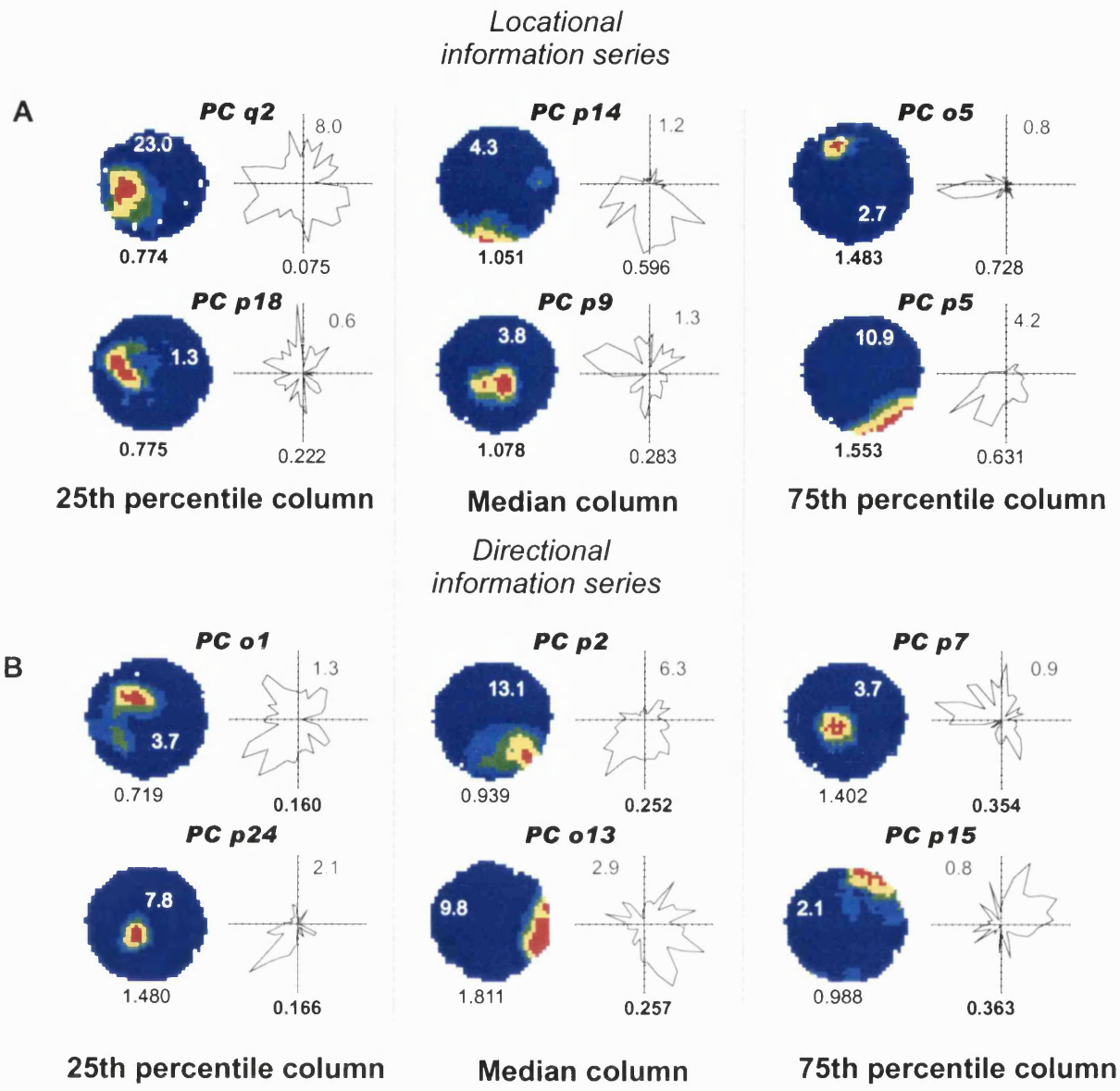


Fig IX.4 Firing rate maps and directional polar plots of 12 representative place cells.

For each cell, the locational firing rate map (left), and the directional polar plot (right) are shown. The number in white in the rate map, and that at the top of the polar plot, denote the Locational and Directional peak firing rate respectively, in Hz. The appropriate Burgess Information measure score is also shown (underneath the map or plot).

A) Place cells arranged in order of increasing values of the Burgess Locational information measure.
B) Place cell arranged in order of increasing values of the Burgess Directional information measure.

For each category, the left hand column shows the cells lying on the 25th percentile, the middle column shows the cells lying on the median, the right hand column shows the cells lying on the 75th percentile, along the spatial informational dimension in question.

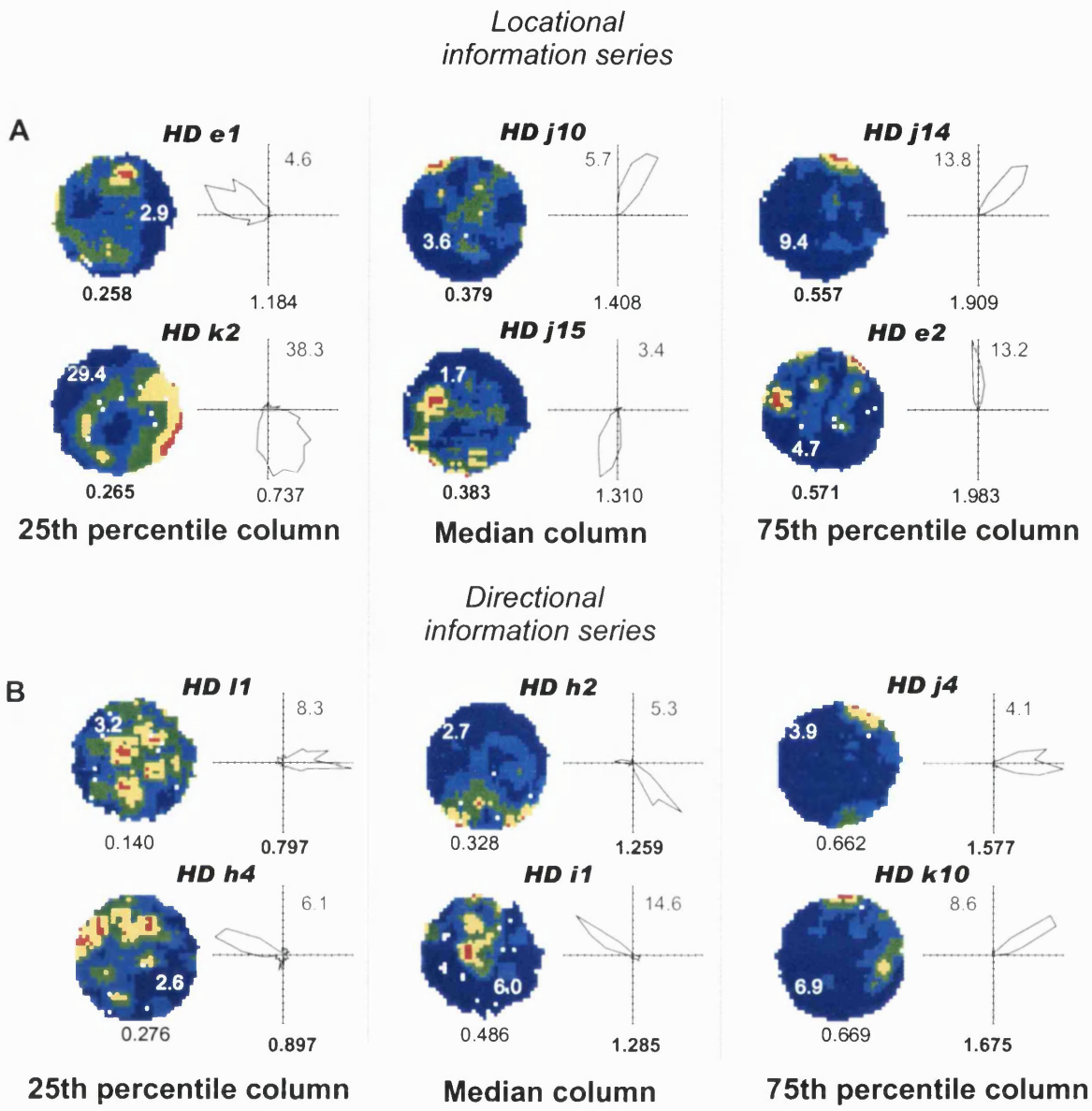


Fig IX.5 Firing rate maps and directional polar plots of 12 representative Head direction cells.

For each cell, the locational firing rate map (left), and the directional polar plot (right) are shown. The number in white in the rate map, and that at the top of the polar plot, denote the Locational and Directional peak firing rate respectively, in Hz. The appropriate Burgess Information measure score is also shown (underneath the map or plot).

A) Head direction cells arranged in order of increasing values of the Burgess Locational information measure.
B) Head direction cells arranged in order of increasing values of the Burgess Directional information measure.

For each category, the left hand column shows the cells lying on the 25th percentile, the middle column shows the cells lying on the median, the right hand column shows the cells lying on the 75th percentile, along the spatial informational dimension in question.

"compactness" of the fields. This measure was preferred to the spatial coherence measure introduced by Kubie et al (1990) because at the smoothing levels used in this study spatial coherence shows a ceiling effect.

Locational field size was defined as the number of bins (not necessarily contiguous) where firing was higher than 1 Hz and expressed as a percentage of the total number of visited bins. Directional firing range is expressed in degrees and is calculated as the extent of arc in which firing is half the maximal firing rate.

Cells were assigned to the three groups (PC,TD and HD) on the basis of *a priori* considerations. The purpose of the statistical analysis presented is to provide some quantitative justification for this sorting.

Results

Table I summarises the results, providing the mean ± SEM for the parameters selected across the three cell types. Fig IX.6-7 show the boxplots and bar graphs of PC, TD and HD distributions for each spatial measure considered.

A one-way multivariate analysis of variance, with cell type as the between-subjects factor, was carried out on the 10 measures of spatial selectivity used. The overall multivariate analysis for group was significant [$F(30,384) = 27.74$ $p < 0.0001$] allowing inspection of the univariate analyses of variance (ANOVA) for the 10 dependent variables. Newman-Keuls comparisons (NK), which control for multiple comparisons, were the *post-hoc* tests employed in all cases.

In general, the results show that the locational and directional selectivity of TD cells are intermediate between those of HD and PC cells. Overall, TD cells show values which are closer to those found in HD cells on most of the locational measures considered. A possible explanation for this pattern of results will be given in the discussion section.

Locational firing properties

The overall firing rates for PC, TD and HD were 1.05 (±0.20) Hz, 1.74 (±0.17) Hz and 1.77 (±0.43) Hz respectively, and there was no significant difference between them [$F(2,135) = 1.98$, $p = 0.142$].

Locational selectivity is high in PC cells (8.78 ± 0.59 Hz) and lower for TD and HD cells (TD 5.37 ± 0.35, HD 5.03 ± 0.36; see Table I and fig IX.6). The main effect of cell type was significant [$F(2,135) = 21.49$, $p < 0.001$], *post-hoc* tests revealed that PC cells differ significantly from TD and HD cells (NK, $p < 0.01$), while there is no significant difference between the TD and HD cells' values.

A very similar pattern is apparent for all the locational field measures shown in fig IX.6. There was a main effect of cell type for Skaggs locational information [$F(2,135) = 61.96$, $p < 0.001$], Burgess locational information [$F(2,135) = 58.40$, $p <$

0.001], IESA [$F(2,135) = 68.29$, $p<0.001$], and place field size [$F(2,135) = 15.55$, $p < 0.001$]. For all these measures *post-hoc* tests showed that PC cells were significantly different from both TD and HD cells (NK, $p < 0.01$), while TD and HD cells did not differ from each other. Only for the Burgess locational information measure does the difference between TD and HD reach significance (NK, $p < 0.05$).

Thus, TD and HD cells show lower values of locational information and spatial field "compactness" (as measured by IESA) relative to place cells. The difference in locational field size goes in the opposite direction: PC fields cover on average 22% (±2%) of the environment and are much smaller than those of TD (49 ± 3 %) and HD cells (43% ± 4%). The fact that TD place fields are on average bigger than HD place fields is counterintuitive and deserves some comment. As it is apparent from fig IX.5, many HD cells show localised firing patterns in the cylinder. These "spurious" place fields emerge due to sampling biases. This problem will be discussed in more detail in the next chapter, but in brief, let us consider the firing pattern of cell HDj14. This cell's preferred direction is north-east. This means that whenever the animal points his head north-east the cell's firing reaches its highest rate. The cell also shows a defined place field, against the north-eastern portion of the cylinder's wall. This place field is very likely the result of a sampling artefact: whenever the rat is in the north-east region of the cylinder, it will often face north-east and cannot face south-west (because of the physical constraints that the rat body poses). Thus, in the north-east portion of the cylinder, head direction sampling is highly biased towards the cell's preferred direction, the cell fires maximally there and a "spurious" place field emerges. This problem will be addressed in the next chapter, where a new correcting algorithm will be introduced and applied to all cell types, to distinguish between real and artefactual firing correlates.

In summary, based on this analysis, the locational selectivity of TD cells is lower than that of place cells and more similar to that of HD cells. For almost all the measures of locational firing considered (except locational field size) TD mean values are intermediate between those of PC and HD cells. The sampling bias discussed above could be responsible for the lack of significant difference in the locational properties of TD and HD cells.

Locational field position

The spatial distribution of place fields within the cylinder for the three cell types is shown in fig IX.8 A. The position of the highest firing rate bin is marked in green for PC, blue for TD and red for HD cells. While the green dots are scattered uniformly across the circle, the blue dots are more dense towards the outer two-thirds of the cylinder and the red dots are almost exclusively found against the wall of the cylinder.

This pattern is also evident in the boxplots shown in fig. IX.8 B which represent the distribution of the distances from the wall of the locational peaks for PC, TD and HD cells expressed as a ratio of distance from the wall (a value of zero indicates that the locational peak is in the centre of the cylinder, 1 means it is on the wall). Locational fields of place cells are symmetrically distributed across all values (0.49 ± 0.03), TD cells' distribution is more skewed towards the wall (0.57 ± 0.03) and this effect is even more dramatic for HD cells (0.67 ± 0.02).

Table I

			Positional measures						Directional measures				
cell type	Number of cells	Overall firing rate (Hz)	Peak firing rate (Hz)	Locational selectivity	Skaggs info	Burgess info	IESA	Field size (>1Hz)	Peak firing rate (Hz)	Directional selectivity	Directional range (deg)	Skaggs info	Burgess info
PC	46	1.05 ± 0.20	7.20 ± 0.88	8.78 ± 0.59	1.39 ± 0.09	1.12 ± 0.06	0.49 ± 0.012	0.22 ± 0.02	2.43 ± 0.35	2.79 ± 0.15	116.72 ± 10.47	0.32 ± 0.04	0.32 ± 0.03
TD	46	1.74 ± 0.17	8.34 ± 0.81	5.37 ± 0.35	0.60 ± 0.04	0.57 ± 0.04	0.39 ± 0.003	0.49 ± 0.03	7.09 ± 0.67	4.38 ± 0.25	69.85 ± 4.08	0.80 ± 0.06	0.74 ± 0.05
HD	46	1.77 ± 0.43	6.80 ± 1.19	5.03 ± 0.36	0.45 ± 0.04	0.43 ± 0.03	0.37 ± 0.002	0.43 ± 0.04	9.73 ± 1.47	7.29± 0.44	42.17 ± 2.87	1.39 ± 0.09	1.26 ± 0.07

Fig IX.6 Summary of measures of locational firing characteristics of place cells (green), topodirectional cells (blue) and head direction cells (red).

For each variable, box plot is shown on left, and mean and standard error plot is shown on right.

Locational selectivity is the Locational Peak Rate divided by the Overall mean firing rate. Algorithms taken from Skaggs et al (1993) and Burgess et al (in preparation) have been used to determine locational information. The units for both Information scores are bits per spike.
IESA is the inverse of the entropy of the spatial autocorrelation (Burgess et al, in preparation).
Field size is expressed as the percentage of the total environment where firing rate was above 1 Hz.

For most measures of locational firing, place cells show the values associated with the highest degree of location-specific firing, head direction cells show the lowest, and topodirectional cell values are intermediate between these two types (though closer to those of head direction cells). The exception is the field size measure, where the largest fields are shown by topodirectional cells. Note that the locational information of topodirectional cells is significantly larger than that of head direction cells on the Burgess Locational Information score.

** denotes a significant difference between groups at $p < 0.01$.
* denotes a significant difference between groups at $p < 0.05$.

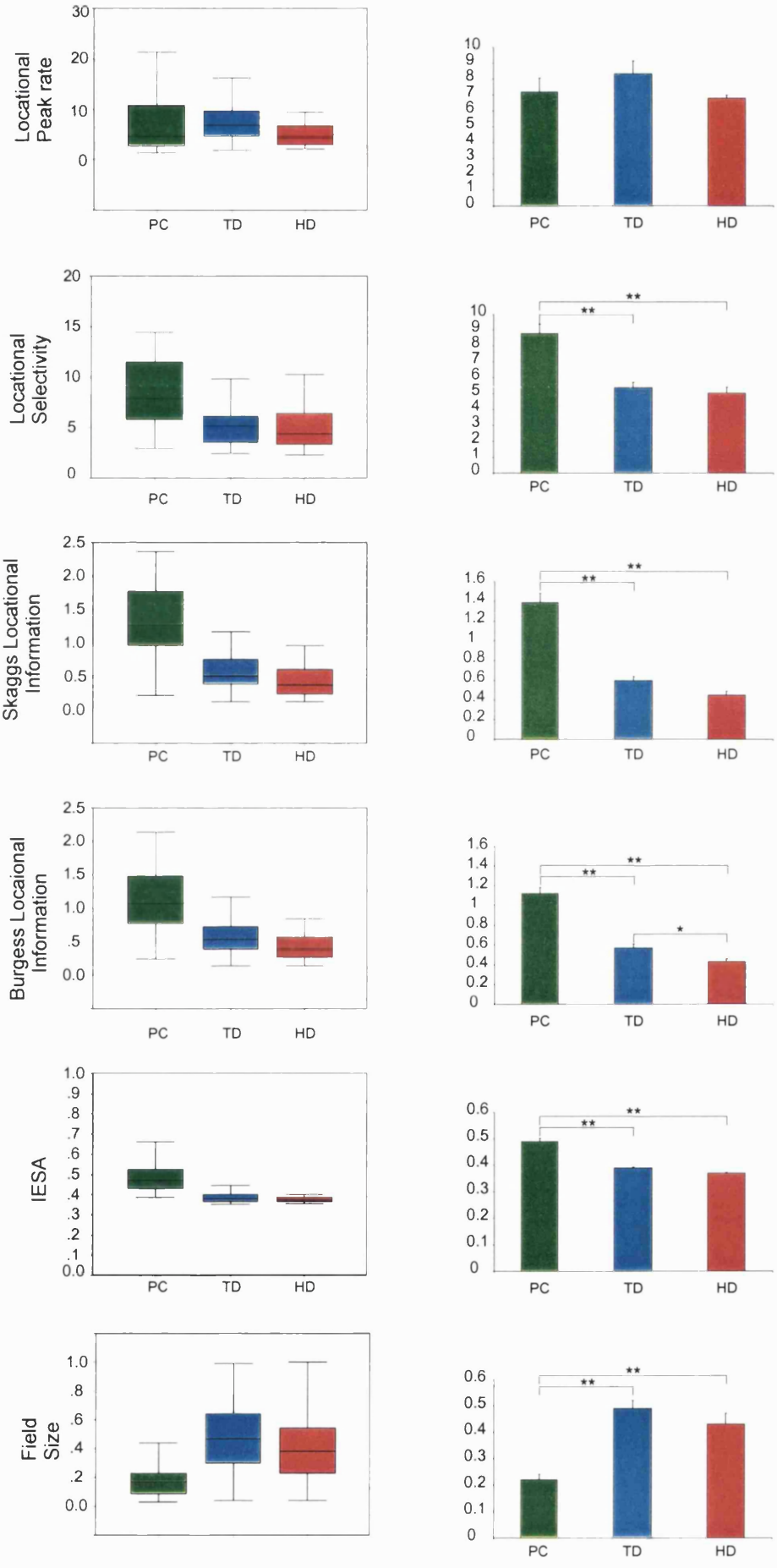

Locational Peak rate
Locational Selectivity
Skaggs Locational Information
Burgess Locaional Information
IESA
Field Size
PC
TD
HD
**
*

Fig IX.7 Summary of measures of general and directional firing characteristics of place cells (green), topodirectional cells (blue) and head direction cells (red).

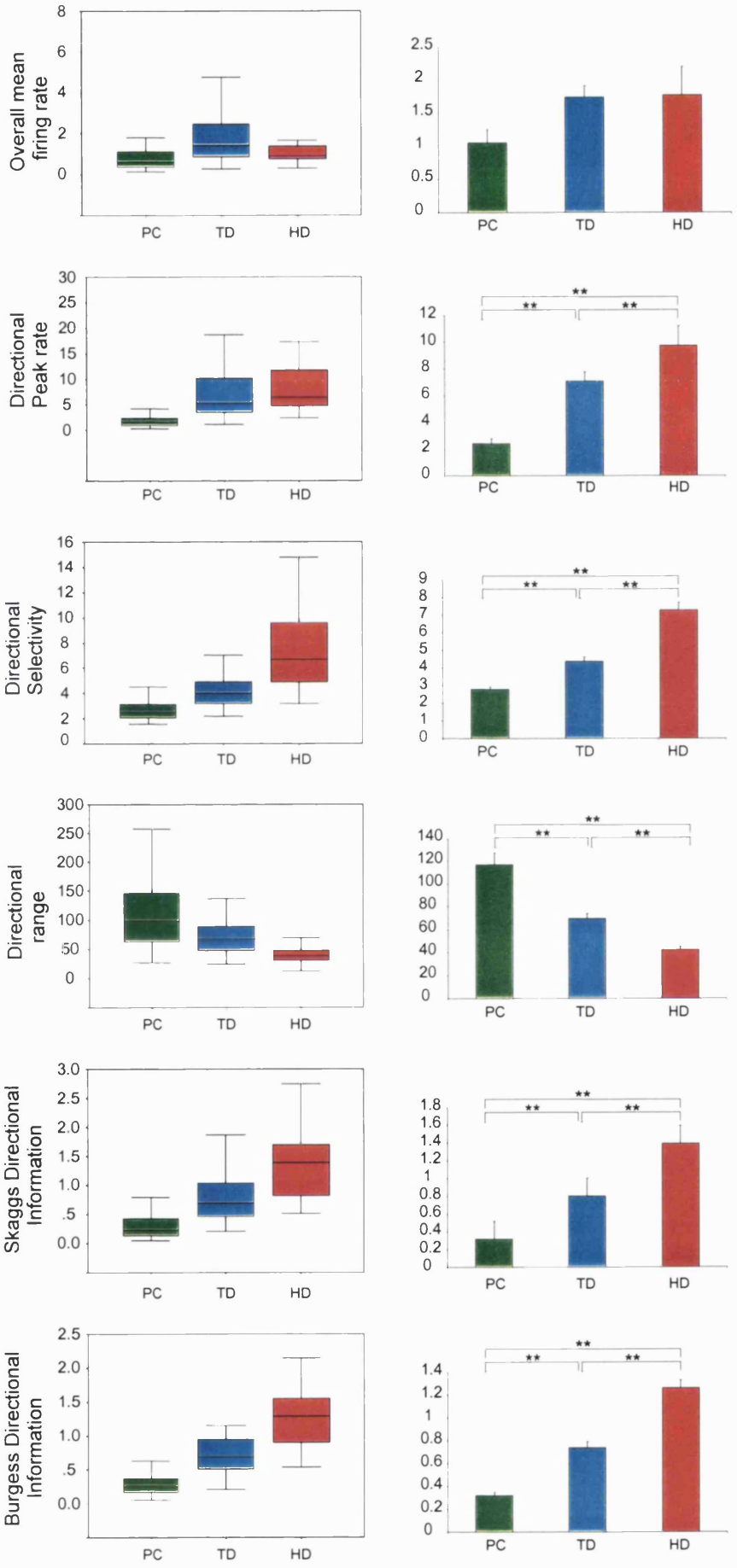
For each variable, box plot is shown on left, and mean and standard error plot is shown on right.

Directional selectivity is the Directional Peak Rate divided by the Overall mean firing rate. Directional range is the extent of arc in which firing is over half the maximal firing rate.

Algorithms taken from Skaggs et al (1993) and Burgess et al (in preparation) have been used to determine directional information. The units for both Information scores are bits per spike.

Note that for all the five measures of directional firing, place cells show the values associated with the lowest degree of directionality, head direction cells show the highest, and topodirectional cell values are intermediate between these two types.

** denotes a significant difference between groups at $p < 0.01$


Overall mean firing rate
Directional Peak rate
Directional Selectivity
Directional range
Skaggs Directional Information
Burgess Directional Information
PC
TD
HD
**

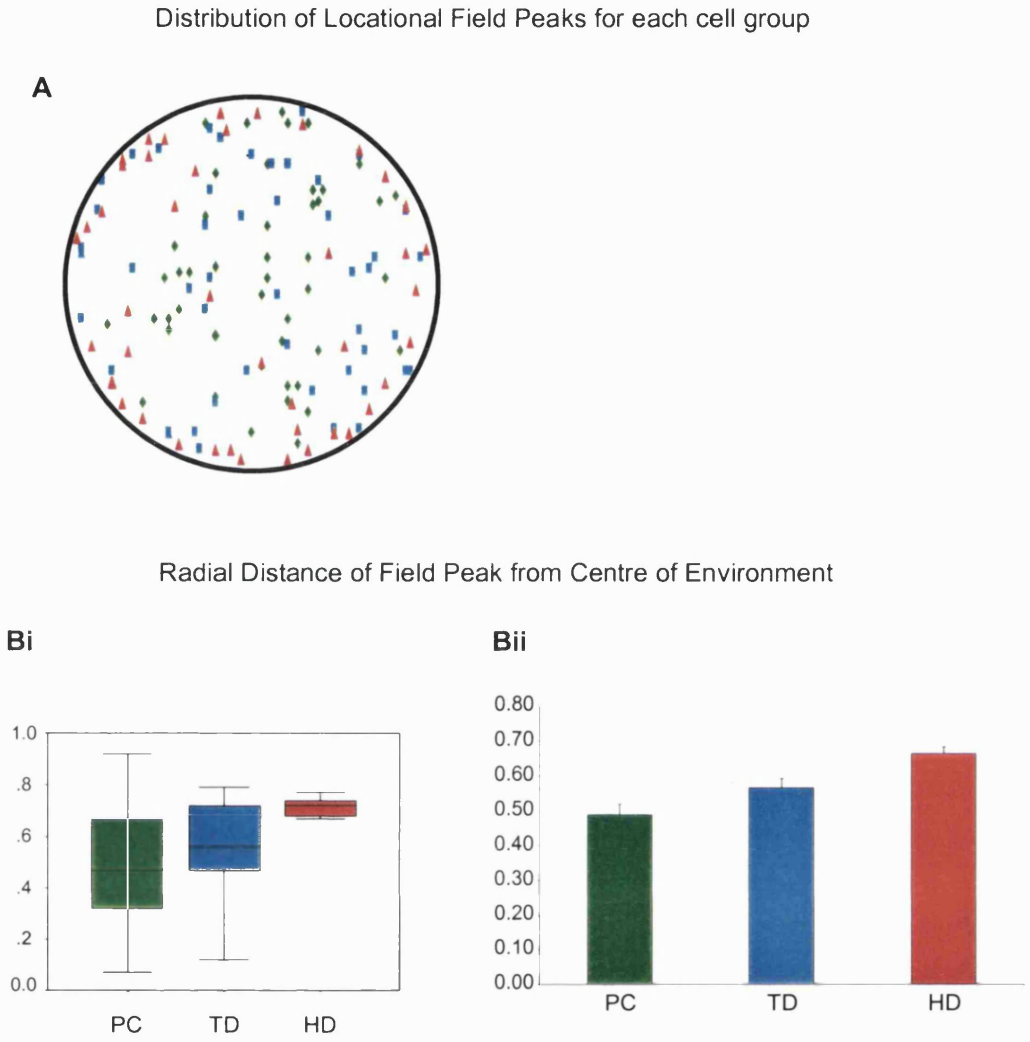


Fig IX.8 Characteristics of Locational Peak preferences in place cells (green), topodirectional cells (blue) and head direction cells (red). There are 46 cells in each group.

A) Distribution of Locational Peaks within the cylinder for each cell group. It can be seen that Head direction cell peaks tend to occur near the edge of the environment, while those of topodirecitonal cells and place cells are more evenly distributed.

B) Box plot (Bi) and Mean and Standard Error Plot (Bii) of Radial Distance of Locational Field Peak from Centre of Environment.

The data in Bi and Bii provide some formal confirmation that head direction cell peaks are preponderantly near the edge of the environment, that place cell peaks are evenly distributed, and that topodirectional cell peaks are intermediate between these two cell types.

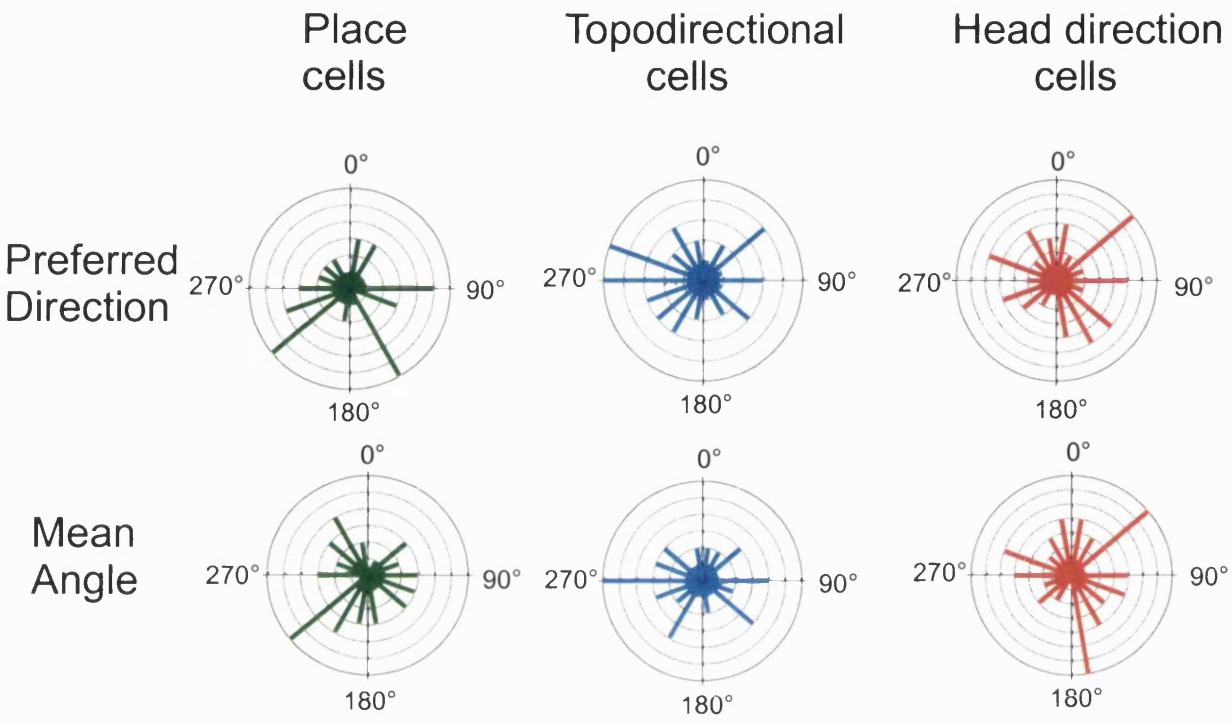


Fig IX.9 Characteristics of directional preferences in place cells (green), topodirectional cells (blue) and head direction cells (green).

For two measures of directional preference (Preferred Direction and Mean Angle), it can be seen that there is no particular angular preference shown by any of the three groups of cells.

The fact that almost all HD place fields are clustered around the edges of the environment is again due to the sampling bias mentioned above and reflects the fact that these locational fields are artefactual. On the other hand, TD place fields distribution is only mildly affected by the sampling problem, indicating that TD place fields are not purely artefacts.

Directional firing properties

The directional measures considered were: directional selectivity, directional firing range and Skaggs and Burgess information measures for directional information. The mean values and SEM for each cell type are summarised in Table I.
All main effects of cell type were significant across all measures tested: directional selectivity [$F(2,135) = 54.95$, $p<0.001$], Skaggs directional information [$F(2,135) = 59.60$, $p<0.001$], Burgess directional information [$F(2,135) = 81.75$, $p<0.001$] and directional firing range [$F(2,135) = 31.67$, $p<0.001$]. *Post-hoc* tests showed that each group was significantly different from each other (NK, $p < 0.01$).
The directional firing properties of TD cells thus lies in between those of PC and HD cells.

A very low, though detectable, directional selectivity was found for PC cells in this study (see Table I and fig IX.7). This residual directionality may well be due to the same behavioural bias mentioned above in accounting for the ostensibly locational selectivity of HD cells.

Preferred direction

The heading angle at which maximal cell activity was recorded (preferred direction) and the mean angle (circular mean of the distribution) were computed for each place, topodirectional and head direction cell.
The distributions of both these measures for each cell type are shown in the form of angular histograms in fig IX.9. It is evident that in all cases the values are scattered uniformly around the 360° range. A Rayleigh test confirmed that for all three cell populations it was not possible to discard the null hypothesis that the angular distributions were uniform (PC preferred direction $p = 0.43$, mean angle $p = 0.67$; TD preferred direction $p = 0.70$, mean angle $p = 0.29$; HD preferred direction $p = 0.28$, mean angle $p = 0.24$).

Moreover, no obvious topographical organisation was apparent: TD cells simultaneously recorded did not show similar preferred directions, very much like what is already known for head direction cells.

Summary and discussion

The results of the statistical analysis performed can be summarised as follows: topodirectional cells show intermediate values between those of place and head-direction cells across a variety of locational and directional firing measures.

These results confirm the intuitive impression that figs IX.1-2 give, that topodirectional firing is modulated by both location and direction.

It is not possible to compare directly the relative strength of these two variables in modulating topodirectional firing. This is mainly because for this analysis, the average number of bins contained in the locational maps (900) was very different from those in the directional plots (36), and the measures of spatial selectivity considered are sensitive to bin number. In particular, all information measures used (Skaggs, Burgess) have been shown to be sensitive to number of bins (Burgess et al, in preparation).

The results of the statistical analysis must be considered with caution because some of the directional and/or locational selectivity of topodirectional cells might be artefactual, emerging from the same sampling biases responsible for the residual directional selectivity detected in place cells, and locational selectivity in head direction cells. This issue will be addressed in the next chapter, where a statistical analysis on the corrected values of the spatial measures considered here will be presented.

The sampling artefacts were reflected by the fact that the peaks of the locational fields of head direction cells were concentrated at the periphery of the cylindrical environment, while those of topodirectional cells were more uniformly scattered across the recording environment (even though not as much as those of place cells). In a similar way to locational peaks, no clustering around a particular value was seen in either the preferred direction or the mean directional angle for topodirectional cells (and head direction cells).

In summary, the position of topodirectional cells locational fields, and their preferred directions are homogeneously distributed across the locational and directional spatial spectra.

In addition to this, no obvious topographical arrangement of TD cells was detected: TD cells recorded simultaneously or sequentially along one electrode penetration did not show spatially related locational or directional properties.

Chapter X

Locational and directional contributions to the firing of topodirectional cells

The firing of topodirectional cells appears to be modulated by both the animal's position and head-direction. The main problem that this chapter will address is that due to inhomogeneous sampling of positions and head directions the contribution of these two behavioural variables to topodirectional cell firing is difficult to judge.

The intrinsic behavioural constraints posed by the geometry of the animal's body and the environment it is traversing create dependencies between these two variables that can lead to artefactual results. For example, as it has been suggested in the previous chapter, sampling biases cause place cells to show an apparent directional modulation of their firing rates and, conversely, the firing of head direction cells appears to be modulated by the location the animal occupies. This is because, for place cells, if the animal samples more frequently some head-directions whenever he is in the place field of a given place cell, a spurious head direction response will be attributed to that cell (see fig IX.4). Similarly, if some places are sampled more frequently when the rat is facing in the preferred direction of a given head-direction cell, the cell will be attributed a "secondary", though artefactual, locational component to its firing rate (see fig IX.5).

This problem makes it hard to assess the relative strength of the locational and directional components of topodirectional cells' firing.

One previous attempt has been made to address this issue in relation to place cells. Muller et al (1994) introduced a method, the "Distributive hypothesis" procedure to quantify the problem of the confound created by the influence of head direction on the firing of place cells. The authors calculated the directional firing that one would obtain under the null hypothesis that place cells only code for the animal's location in space and that the only directional influence detected is due to the sampling bias discussed above. They then tested whether the observed directional tuning for the cell differed from the one calculated under the assumption that the effect of direction was purely artefactual. They did so by calculating a "distributive ratio" whose value is zero for a perfect prediction (indicating that the assumption is correct and that after all place cell firing is only influenced by the animal's location), while high values of this ratio would indicate a poor prediction (indicating that head direction accounts for some of the variability in the cell's firing rate).The computational details of this method are described in the Methods section of this thesis.

Muller et al (1994) successfully showed that the directional correlates of place cells' firing is largely artefactual and suggested that the same algorithm might be applied to head direction cells, by swapping the place and direction terms.

The distributive hypothesis method is an interesting diagnostic tool, that helps identify possibly artefactual modulations of a cell firing. It does not, however, allow one to estimate the relative effect of location and direction on the firing of a cell genuinely modulated by both variables.

In this chapter the distributive hypothesis method will be applied to compute the locational and directional distributive ratios for place, topodirectional and head direction cells, to understand how much the locational fields of the cells can be predicted solely on the basis of head direction and vice-versa.

In an attempt to disentangle the possible contribution of location and head direction to PC, TD and HD cells' firing, a new computational approach developed by Neil Burgess will be used (Burgess et al, in preparation). In this approach, the firing rate of a cell is modelled as the product of independent functions of the rat's location and direction. The separate dependencies on location and direction are then estimated using a maximum likelihood approach (see Methods section p.72). This provides a way of "correcting" the artefacts introduced by the inhomogeneous sampling of the behavioural variables in question to the cells' firing rates. The algorithm goes under the name of "PxD" (place by direction), and the reader is referred to the methods section of this thesis for a description of its computational details.

Methods

Cell samples were composed of 17 PC, 17 TD and 17 HD cells.
TD cells were recorded from 5 rats (rat a = 7 cells, b = 2, d = 2, f = 4, g = 1).
HD cells were recorded from 8 rats (rat e = 2 cells, f = 3, h = 1, i = 1, j = 3, k = 3, l = 3, m = 1). These cells were all taken from the larger samples used in the previous chapter.

Note that in some animals both TD and HD cells were recorded (rats a and f). In one occasion one TD cell (TDf4) and one HD cell (HDf6) were simultaneously recorded. On a separate occasion two TD cells (TDf5-6) and one HD cell (HDf6) were simultaneously recorded.

PC cells were recorded by Colin Lever and Tom Wills, from CA1, in 6 rats (o = 1 cell, p = 7, r = 3, s = 3, t = 1, u = 2), including some which were not present in the sample used in the previous chapter.

Data was collected over a total of 20 minutes recording for each cell. Data was collected from the cylindrical enclosure, over 2 trials, each 10 minutes long. For this analysis more data (20 vs 8 minute recording sessions) was taken for each cell. This was done to ensure extensive sampling of both location and direction.

Cells were closely matched for number of spikes, resulting in a mean overall firing rate value of 1.17 Hz (±0.13) for PC, 1.17 Hz (±0.17) for TD and 1.17 Hz (±0.13) for HD cells. This was done to control for the strong positive correlation that was noticed between both the locational and the directional distributive ratios and number of spikes across all cell types (data not shown).

The locational firing rate maps presented in this chapter contain an average of 70 bins, while the directional maps contain 64 bins. These binning values were carefully chosen in order to allow a fair comparison between the place and directional cells' responses. This is because, as mentioned in the previous chapter, the information measures used to quantify the spatiality of the cells' firing are highly dependent on number of bins (Skaggs and Burgess locational and directional information measures, spatial coherence). The same considerations apply to both the directional and locational distributive ratios and the PxD algorithm. In both procedures (distributive hypothesis and PxD), when the locational firing of the cells is analysed, the environment is divided into 70 locational bins, each of which contains 64 directional bins. Symmetrically, when the directional firing of the cells is taken into consideration, the polar plots are divided into 64 bins, each containing 70 locational bins. The choice of 70 locational and 64 directional bins was made to ensure that the two variables (location and direction) would be treated in the most similar way possible. No smoothing was applied to either the locational or directional maps.

Results

Distributive Hypothesis Procedure

Both locational and directional distributive ratios were computed for the cells included in this analysis. As mentioned above, distributive ratios measure the goodness of fit between a predicted and an observed firing distribution. In particular the locational distributive ratio measures the similarity between the observed locational map and the one predicted under the assumption that all the locational firing is artefactual. If the assumption is met, the prediction is perfect and the value that the locational distributive ratio takes is zero. Departures from zero indicate that not all the locational response of the cell can be accounted for on the basis of inhomogeneous sampling.

Similarly, low values of the directional distributive ratio indicate that the directional tuning of the cell is mostly artefactual, while high values mean that the cell's firing is genuinely controlled by head direction.

We would expect that place cells have low values of directional distributive ratio and high values of locational distributive ratio, HD cells high values of directional distributive ratio and low values of locational distributive ratio. TD cells' values are expected to be intermediate between them, since both location and head direction have an influence on the firing of these cells. Thus, neither the locational nor the directional firing patterns of these cells can be fully predicted on the basis of the other spatial variable.

Fig X.1 confirms that our predictions are correct, showing the distributions of the locational and directional ratios for the three cell populations. The mean values for the locational distributive ratio were: 0.55 (± 0.04) for place cells, 0.31 (±0.03) for TD cells, and 0.25 (±0.02) for HD cells.

The mean values for the directional distributive ratio were: 0.16 (±0.01) for place cells, 0.38 (±0.03) for TD cells and 0.44 (±0.03) for HD cells.

The value of the directional distributive ratio for place cells is very similar to the one reported by Muller et al (1994) for their sample of 16 place cells (0.19 ± 0.03), confirming the robustness of the finding.

It is also apparent that the directional distributive ratio for TD cells is somewhat higher than the locational one, indicating that the directional firing of the cells is more robust than their locational firing.

An ANOVA revealed that there was a significant effect of cell type on both the locational [$F(2,48) = 28.40$, $p < 0.001$] and directional [$F(2,48) = 32.53$, $p < 0.001$] distributive ratios. *Post-hoc* tests showed for both distributive ratios, that PC values differed significantly from both TD and HD values (NK, $p < 0.01$), but that the difference between TD and HD did not reach significance (NK, $p > 0.05$).

Five examples of PC, TD and HD cells are shown in fig X.2 A-C. Locational firing rate maps and polar plots are shown for each cell alongside the predicted map and predicted directional tuning. Numbers in boxes represent the values of the locational distributive ratio (next to the predicted locational firing map) and the directional distributive ratio (next to the predicted polar plot). For PC cells, locational distributive ratio values are generally high, and the predicted maps (third column) differ greatly from the observed ones (first column). The predicted and observed directional profiles are almost always completely superimposable (and consequently the values of the directional distributive ratio are very low). For HD cells the converse pattern is true: in general, there is good agreement between the predicted and observed locational maps for HD cells (see HDh3 and HDk6), while this is not the case for the polar plots.

As suggested by the results presented above, the profile for TD cells appears to be intermediate. The predicted locational maps capture some elements of the observed maps (see cell TDd2, TDf5 and TDf6 for the clearest examples). Similarly, the predicted polar plots, in some cases, partly resemble the observed ones (see TDf5 and TDa28).

The fact that for TD cells the mean value of the locational distributive ratio is lower than that of the directional distributive ratio (see fig X.1 and Table 2) suggests that these cells are more strongly modulated by head direction than by location, as it has also been shown by the statistical analysis.

PxD

Fig X.2 also illustrates the effect the PxD algorithm has on both the locational and directional maps of five examples of PC, TD and HD cells. The leftmost column shows the "raw" locational maps (P,D), while maps in the second column refer to locational data corrected for sampling biases, using the PxD algorithm (PxD). Similarly, the uncorrected and corrected polar plots are marked P,D and PxD respectively and are shown side by side, in the fifth and sixth columns, to allow visual comparison.

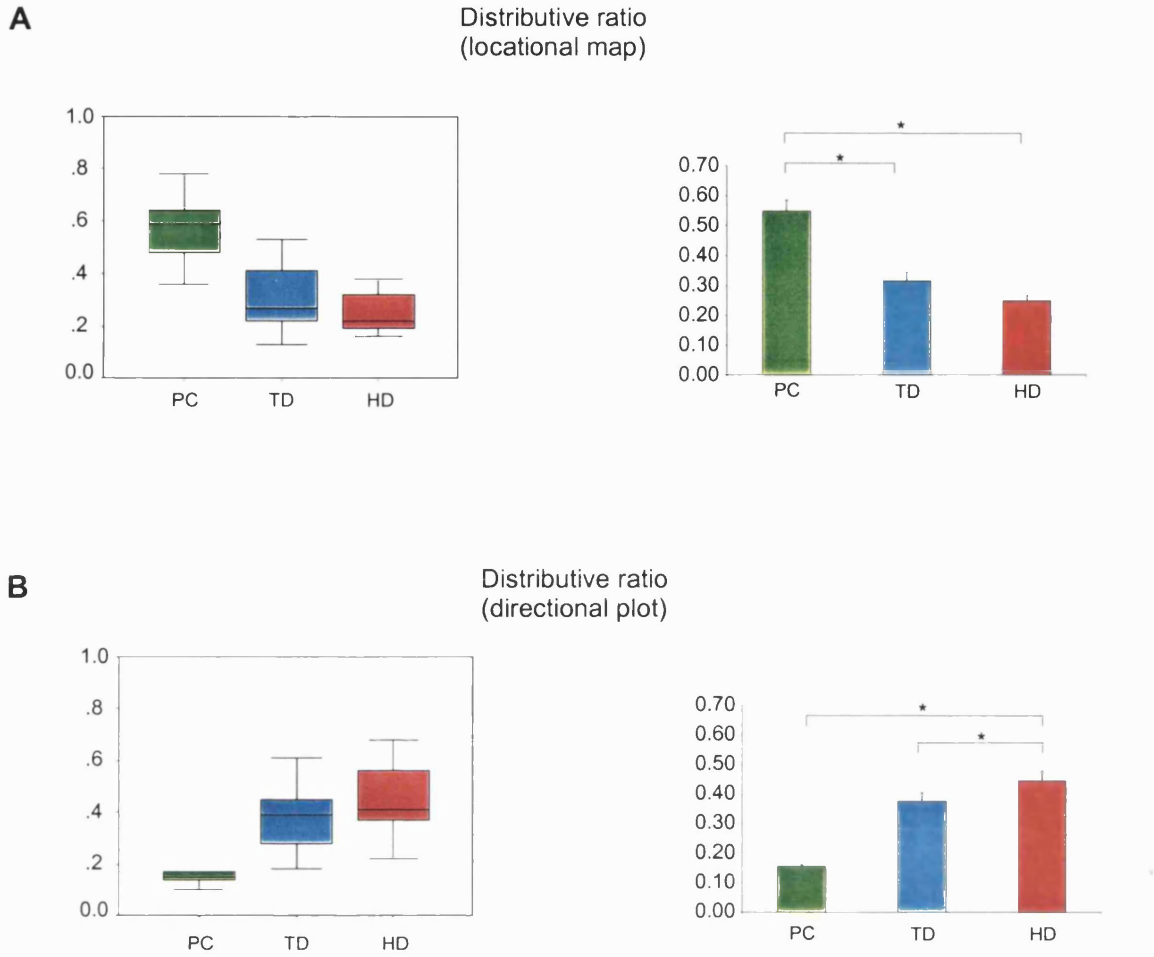


Fig X.1 Summary of descriptive statistics of the Distributive Ratio values in each cell group for locational firing (A) and directional firing (B).

A) Box plot (left) and mean and standard error plot (right) of the Distributive ratio for locational firing. This measures the degree to which the locational firing of the cell can be predicted by its pattern of directional firing. A high value for a cell (closer to 1) indicates robust location-specific firing which is not well predicted by its directional firing.

B) Box plot (left) and mean and standard error plot (right) of the Distributive ratio for directional firing. This measures the degree to which the directional firing of the cell can be predicted by its pattern of locational firing. A high value for a cell (closer to 1) indicates robust directional firing which is not well predicted by its locational firing.

Place cells show values associated with the most robust locational firing and least robust directional firing. Head direction cells show values associated with the most robust directional firing and least robust locational firing. Topodirectional cells show values intermediate between these two cell groups, though closer to those of head direction cells.

An asterisk (*) denotes a significant difference between groups at $p < 0.05$.

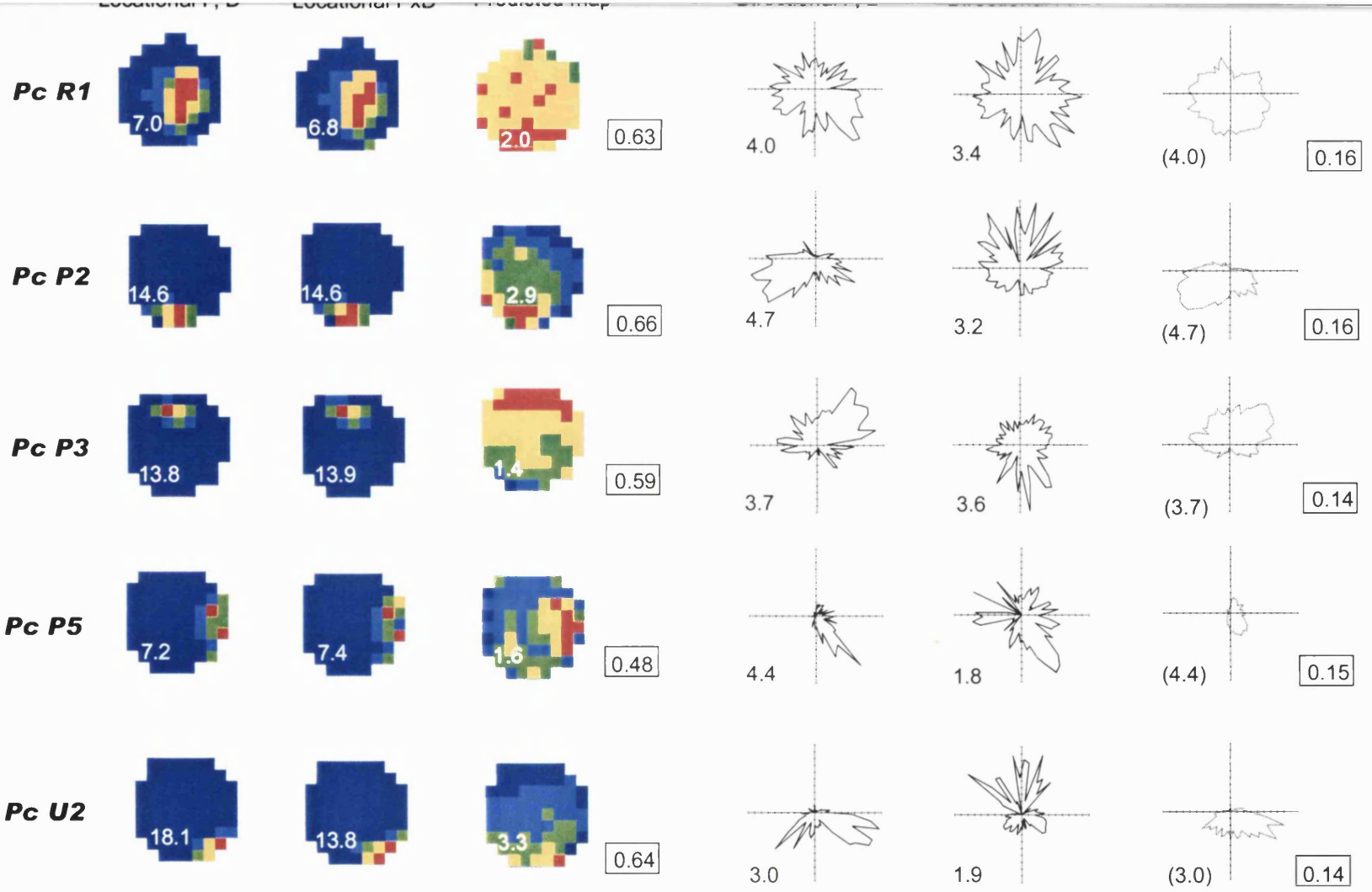


Fig X.2 A Influences of locational and directional variables on place cell firing seen in five place cells.

Locational firing rate maps are shown in left three columns, and directional polar plots in right three columns. In each category of locational and directional firing, the left column (P, D) shows *uncorrected* maps (plots), the middle column (PxD) shows maps (plots) calculated by applying the maximum likelihood procedure for spatial bias of Burgess et al, and the right column shows maps (plots) predicted by the distributional hypothesis algorithm of Muller et al (1994), with the value of the distributional ratio shown in square box bottom right of each map (plot). Place cells' locational correlates are unaffected by PxD model, and not well predicted by their directional firing. Place cells' directional correlates can be significantly altered by PxD model (see e.g. PcP2, 3 & 5), and reasonably well predicted by their locational firing (see e.g. PcP2 &3).

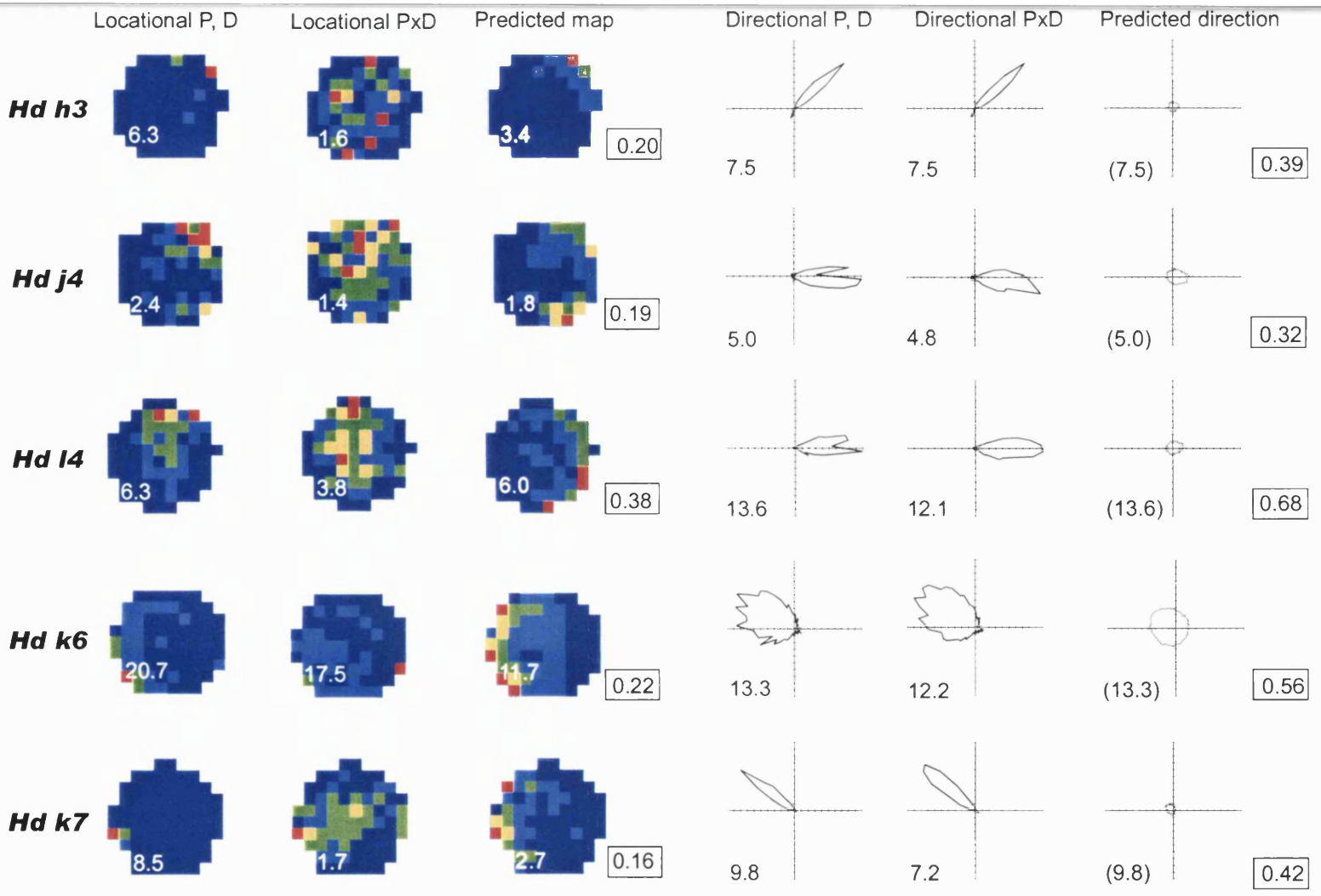


Fig X.2B Influences of locational and directional variables on head direction cell firing seen in five head direction cells.

Conventions are the same as in Fig X.2A.
Head direction cells' locational correlates can be significantly altered by PxD model (e.g. Hdh3 & Hdk7), and can be reasonably well predicted by their locational firing (e.g. Hdh3, Hdj4). Head direction cells' directional correlates are unaffected by PxD model, and not well predicted by their locational firing.

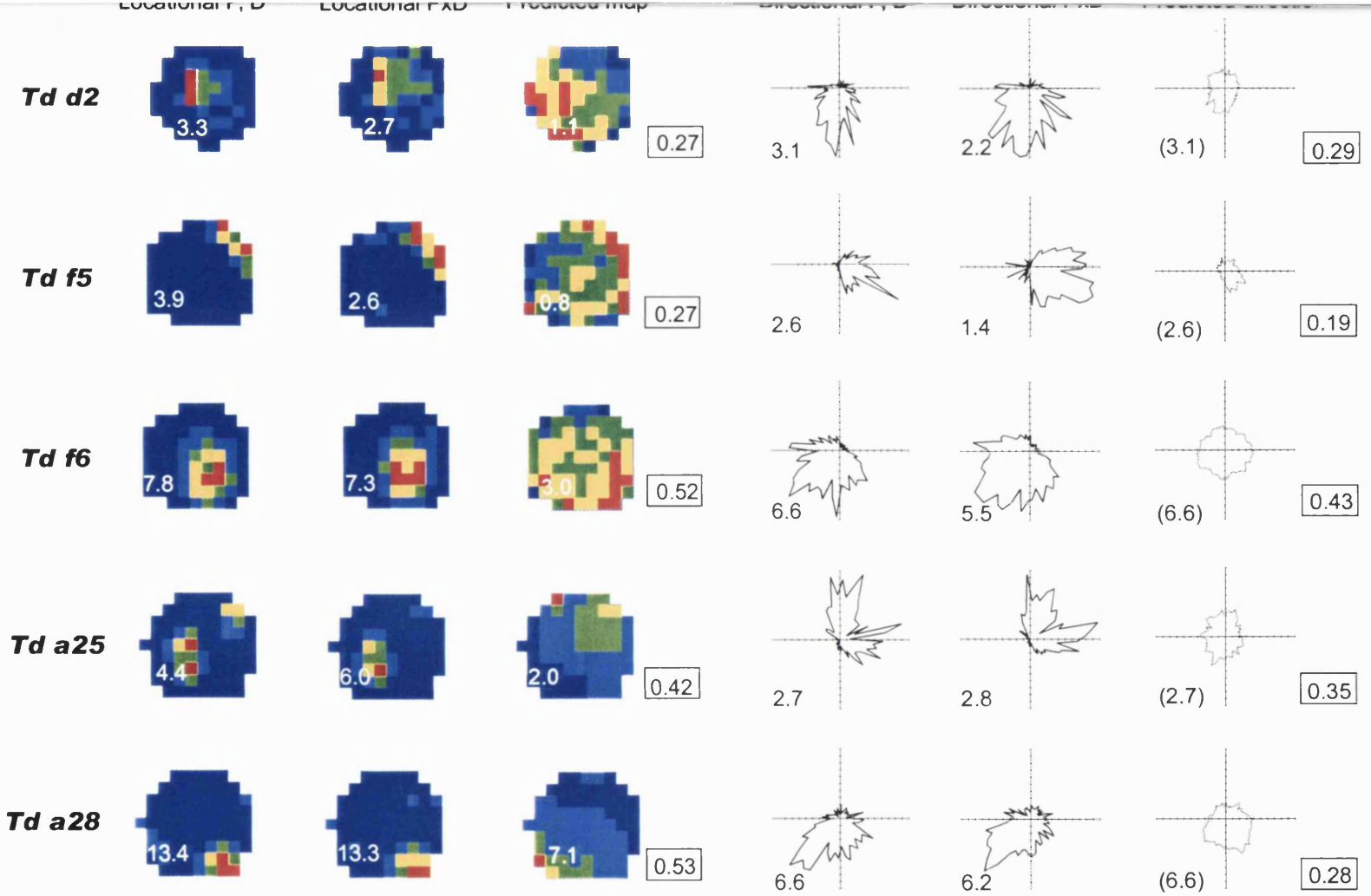


Fig X.2C Influences of locational and directional variables on topodirectional cell firing seen in five topodirectional cells.

Conventions are the same as in Fig X.2A.

Topodirectional cells' locational correlates are largely unaffected by PxD model (though see Tda25 for reduction of apparently spurious locational field), and not well predicted by their directional firing. Topodirectional cells' directional correlates are largely unaffected by PxD model (though see Tdd2 & Tdf5 for some widening the angle of strong directional firing), and not well predicted by their locational firing (except perhaps Tdf5). As such, under two different techniques for removing spatial biases, topodirectional cells locational correlates behave somewhat similarly to those of place cells, while their directional correlates behave somewhat similarly to those of head directional cells.

It is clear that the PxD correction does not really affect the place fields of place cells, confirming that the locational modulation of these cells' firing is robust and not a result of behavioural biases. The effect of the PxD correction on the directional fields of place cells is dramatic. Cell PCP5 is perhaps the best example; its uncorrected polar plot (P,D) shows a clearly directional modulation in the south-south-eastern direction. This directional field disappears altogether after the PxD algorithm is applied, indicating that it was artefactual. In this case, when the rat was in the place field of the cell (the south-eastern portion of the circle, right against the wall) he would have often faced south or south-east, producing the spurious directional field in the uncorrected polar plot.

The effect of the PxD correction on HD cells' maps is the reverse of that seen for place cells: while the directional plots are hardly affected by the correction, the place maps are generally completely changed. For example, cell HDk7 has a place field in the south-western portion of the circle in the uncorrected map and applying PxD induces a dramatic lowering of the peak firing rate of the field (from 8.5 to 1.7 Hz) and its spreading over a larger part of the environment.

The effect of PxD on the locational maps of TD cells is in general a lowering of peak firing rates, while the geometry of the place fields is largely preserved. An exception is cell TDa25, which shows the presence of a main place field in the south western region of the circle and a sub-field against the north-eastern edge of the environment (P,D). Applying PxD removes almost completely this sub-field, increasing the peak firing rate of the main field from 4.4 to 6.0 Hz. This suggests that the sub-field was artefactual, and if we look at the polar plot for this cell it is clear how it could have emerged: the preferred direction of the cell is north-east, and in the north-east portion of the circle the sampling of head direction will be skewed towards this direction, giving rise to a spurious locational sub-field.

The PxD correction on the directional polar plots of TD cells is similar to that described for their locational fields. In general, peak firing rates in the PxD polar plots are slightly lower than the uncorrected ones, while the shape of the directional modulation is left intact (Tda25 and Tda28) or only slightly broadened (TDd2, TDf5 and TDf6).

In summary, the examples presented in fig X.2 show how the PxD algorithm can discriminate and correct for the artefactual modulations seen in the firing of PC, TD and HD cells due to inhomogeneous behavioural sampling. The PxD algorithm is a powerful tool that allows one to disentangle the spurious and real dependencies of firing rate from several behavioural variables.

Statistical analysis

In order to assess the spatial selectivity of PC, TD and HD cells in the uncorrected and corrected conditions (P,D and PxD), nine spatial measures were computed for each cell in each of the two conditions.

The spatial measures used are the same as those described in the previous chapter, the only difference being that as a measure of field "compactness" here the spatial coherence is used instead of the IESA. This is because in this study the locational maps are not subject to smoothing and thus the spatial coherence measure is not subject to a ceiling effect.

The overall firing rate of the cells was not analysed, given that the cells were closely matched along this variable.

Tables II and III offer a summary of the mean and SEM values obtained for each cell type across all the spatial variables computed in the P,D (Table II) and PxD (Table III) conditions. Fig X.3-4 show the boxplots and bar graphs for PC, TD and HD distributions for each spatial variable discussed in each of the two conditions (plain boxes represent values in the P,D condition, boxes with crossed bar those in the PxD condition).

A multivariate analysis of variance, with cell type as the between subject factor and the P,D vs PxD conditions as the within subject factor, was carried out on the 9 measures of spatial selectivity considered. The overall multivariate analysis for cell type [$F(18,82) = 8.11$, $p < 0.001$], P,D vs PxD condition [$F(9,40) = 10.22$, $p < 0.001$], and the interaction between the two factors [$F(18,82) = 3.85$, $p < 0.001$] were all significant, allowing for inspection of all the univariate analyses of variance (ANOVA) for the 9 dependent variables. Newman-Keuls comparisons were the *post-hoc* tests used in all cases on the cell type factor.

Locational firing properties

The locational measures considered were: locational selectivity, Skaggs and Burgess locational information, spatial coherence and place field size.

The main effects of cell type were all significant: locational selectivity [$F(2,48) = 4.80$, $p < 0.05$], Skaggs locational information [$F(2,48) = 70.65$, $p < 0.001$], Burgess locational information [$F(2,48) = 71.02$, $p < 0.001$], spatial coherence [$F(2,48) = 15.12$, $p < 0.001$] and place field size [$F(2,48) = 4.15$, $p < 0.05$].

Post-hoc tests were conducted and revealed that the mean locational selectivity of PC cells was significantly higher than that of HD cells (NK, $p < 0.05$), while no significant difference was found between PC and TD cells or TD and HD cells.

For both locational information measures (Skaggs and Burgess), the mean values of PC cells were higher than those of both TD and HD cells, and the mean values of TD cells were significantly higher than those of HD cells (NK, $p < 0.05$).

For spatial coherence and place field size a different pattern emerged: mean values of PC were significantly higher than those of both TD and HD cells (NK, $p < 0.05$), but no significant difference was detected between TD and HD cells (NK, $p > 0.05$). The mean values of spatial coherence and place field size of TD cells were higher than those of HD cells, even if these differences did not reach statistical significance.

Table II

P,D

			Positional measures						**Directional measures**						
	Number of	Overall firing	Peak firing	Locational	Skaggs	Burgess	Field size		Peak firing	Directional	Skaggs	Burgess	Directional	Locational	Directional
cell type	cells	rate (Hz)	rate (Hz)	selectivity	info	info	(>1Hz)		rate (Hz)	selectivity	info	info	range (deg)	distr. ratio	distr. ratio
PC	17	1.17 ± 0.13	9.66 ± 0.97	9.43 ± 1.30	1.80 ± 0.12	1.45 ± 0.08	0.21 ± 0.02		3.27 ± 0.29	3.16 ± 0.30	0.35 ± 0.07	0.35 ± 0.06	96.53 ± 15.27	0.55 ± 0.04	0.16 ± 0.01
TD	17	1.17 ± 0.17	7.70 ± 1.31	6.91 ± 0.72	0.72 ± 0.08	0.68 ± 0.07	0.34 ± 0.05		6.81 ± 1.18	6.10 ± 0.59	0.97 ± 0.12	0.90 ± 0.09	53.41 ± 7.06	0.31 ± 0.03	0.38 ± 0.03
HD	17	1.17 ± 0.20	6.20 ± 1.08	6.16 ± 0.97	0.46 ± 0.07	0.43 ± 0.06	0.39 ± 0.06		7.55 ± 0.86	8.01 ± 1.13	1.35 ± 0.17	1.23 ± 0.14	47.65 ± 5.24	0.25 ± 0.02	0.44 ± 0.03

Table III

PXD

		Positional measures						**Directional measures**				
	Number of	Overall firing	Peak firing	Locational	Skaggs	Burgess	Field size	Peak firing	Directional	Skaggs	Burgess	Directional
cell type	cells	rate (Hz)	rate (Hz)	selectivity	info	info	(>1Hz)	rate (Hz)	selectivity	info	info	range (deg)
PC	17	1.17 ± 0.13	9.36 ± 0.89	8.94 ± 0.95	1.79 ± 0.12	1.43 ± 0.08	0.21 ± 0.02	2.34 ± 0.19	2.28 ± 0.23	0.17 ± 0.04	0.16 ± 0.03	180.29 ± 23.03
TD	17	1.17 ± 0.17	9.24 ± 2.47	8.00 ± 1.76	0.56 ± 0.09	0.51 ± 0.08	0.40 ± 0.06	5.59 ± 0.83	5.03 ± 0.46	0.88 ± 0.12	0.82 ± 0.09	66.24 ± 8.26
HD	17	1.17 ± 0.20	5.04 ± 1.19	4.05 ± 0.44	0.29 ± 0.05	0.24 ± 0.04	0.42 ± 0.08	6.69 ± 0.72	7.10 ± 0.94	1.28 ± 0.16	1.21 ± 0.12	50.12 ± 5.70

Fig X.3 Summary of measures of locational firing characteristics of place cells (green), topodirectional cells (blue) and head direction cells (red).

For each variable, box plot is shown on left, and mean and standard error plot is shown on right. Within each cell group, left box is uncorrected firing, right box containing diagonal line is firing corrected for spatial bias by the PxD maximum likelihood procedure.

Locational selectivity is the Locational Peak Rate divided by the Overall mean firing rate. Algorithms taken from Skaggs et al (1993) and Burgess et al (in preparation) have been used to determine locational information. The units for both Information scores are bits per spike.
Spatial coherence is a measure of the ordered neighbourliness of locational firing (see Muller et al, 1993).
Field size is expressed as the percentage of the total environment where firing rate was above 1 Hz.

For all measures of locational firing, place cells show the values associated with the highest degree of location-specific firing, head direction cells show the lowest, and topodirectional cell values are intermediate between these two types. The information scores, spatial coherence, and field size of topodirectional cells are closer to those of head direction cells than place cells, but the locational peak rates, and locational selectivity of topodirectional cells are closer to those of the place cells.

Note that the PxD procedure affects head direction cell firing more than place cell firing. Its effect on topodirectional cells is intermediate between these two.

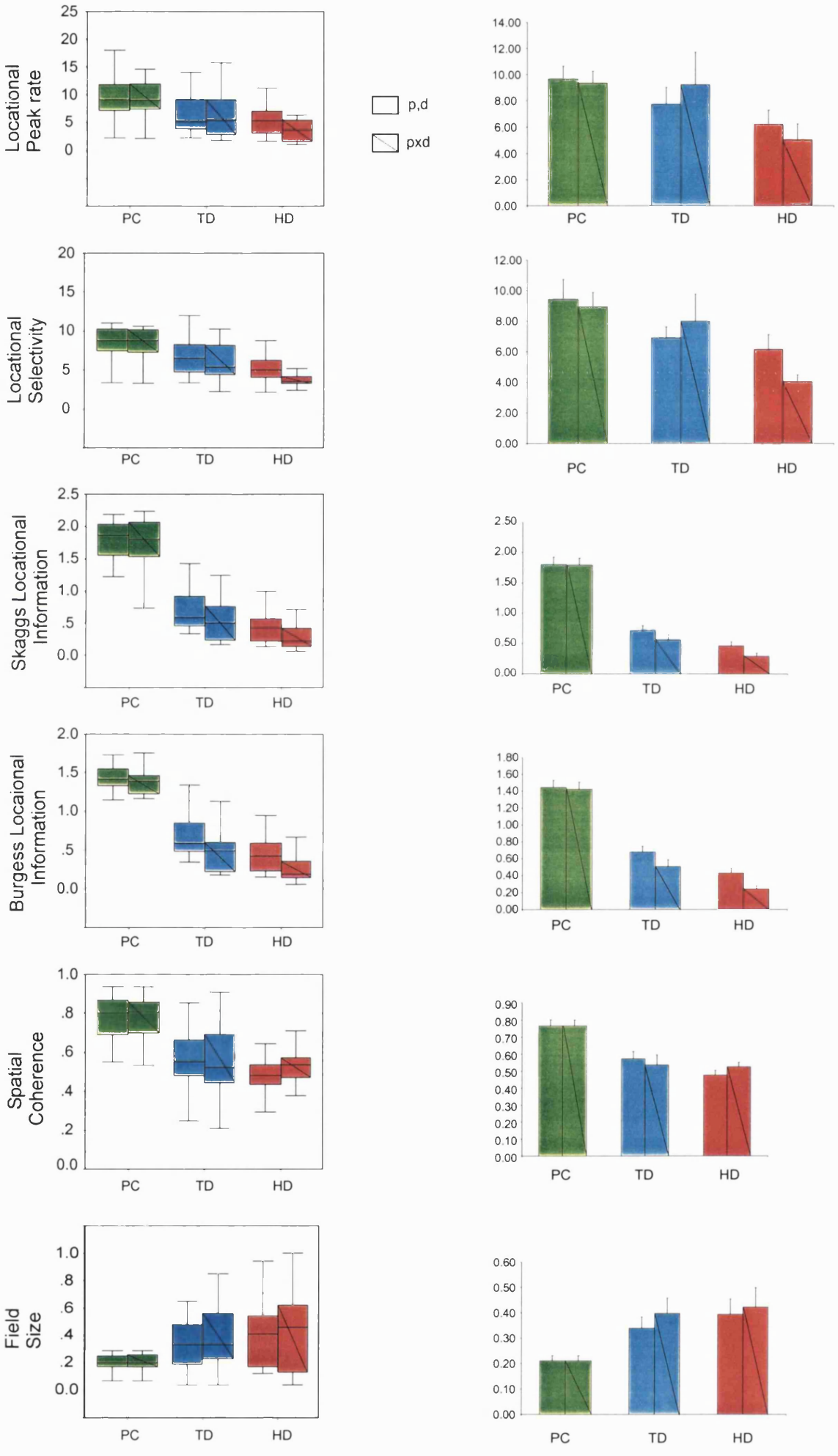

Locational Peak rate
Locational Selectivity
Skaggs Locational Information
Burgess Locaional Information
Spatial Coherence
Field Size
p,d
pxd
PC
TD
HD

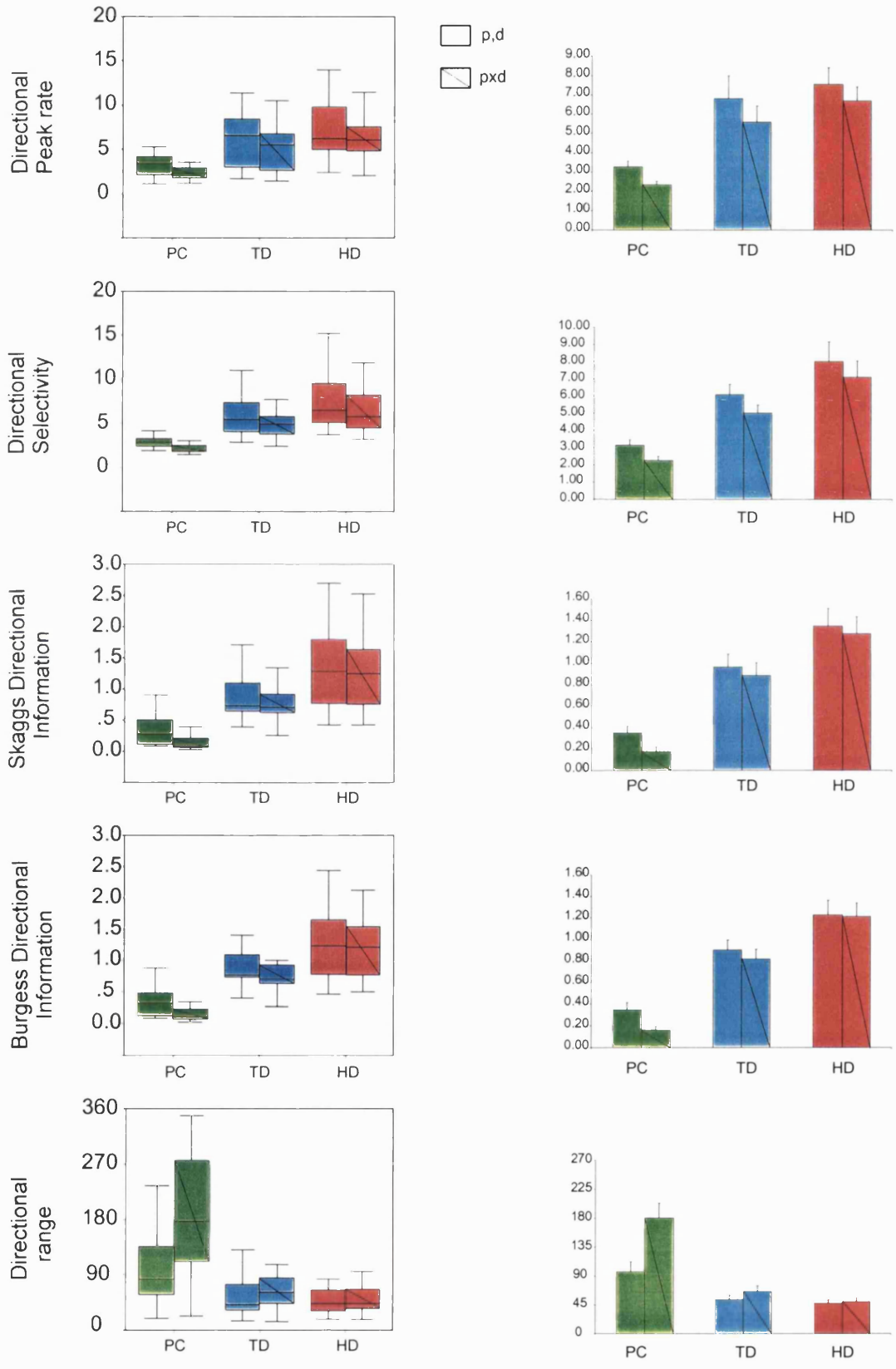
Fig X.4 Summary of measures of directional firing characteristics of place cells (green), topodirectional cells (blue) and head direction cells (red).

For each variable, box plot is shown on left, and mean and standard error plot is shown on right. Within each cell group, left box is uncorrected firing, right box containing diagonal line is firing corrected for spatial bias by the PxD maximum likelihood procedure.

Directional selectivity is the Directional Peak Rate divided by the Overall mean firing rate. Algorithms taken from Skaggs et al (1993) and Burgess et al (in preparation) have been used to determine directional information. The units for both Information scores are bits per spike.
Directional range is the angular spread of firing for which firing was at or above half of the Directional Peak rate of the cell.

For all the five measures of directional firing, place cells show the values associated with the lowest degree of directionality, head direction cells show the highest, and topodirectional cell values are intermediate between these two types.

Note that the PxD procedure affects place cells more than head direction cells and topodirectional cells.


Directional Peak rate
Directional Selectivity
Skaggs Directional Information
Burgess Directional Information
Directional range
p,d
pxd
PC
TD
HD

In summary, for all the locational firing measures considered the values of TD cells were always intermediate between those of PC and HD cells, even though only in some cases these differences were statistically significant. This confirms and supports the results of the statistical analysis applied to the uncorrected maps, which was shown in the previous chapter.

There was a significant effect of PxD correction on: Skaggs locational information [$F(1,48) = 50.86$, $p < 0.001$], Burgess locational information [$F(1,48) = 52.00$, $p < 0.001$] , and place field size [$F(1,48) = 9.89$, $p < 0.05$]. Significance was not reached in the case of the locational selectivity [$F(1,48) = 0.56$, $p = 0.46$], and spatial coherence [$F(1,48) = 0.08$, $p = 0.78$].

Thus, applying the PxD correction resulted in significant changes across only some of the locational variables studied (see Table II and III for a direct comparison between the mean and SEM values in the P,D and PxD conditions).

A significant interaction between cell type and PxD correction was detected for all variables except for locational selectivity [$F(2,48) = 1.85$, $p = 0.168$] and spatial coherence [$F(2,48) = 2.61$, $p = 0.084$]. F and p values for the significant interactions were: Skaggs locational information [$F(2,48) = 9.77$, $p < 0.001$], Burgess locational information [$F(2,48) = 9.08$, $p < 0.001$] , place field size [$F(2,48) = 3.46$, $p < 0.05$].

To give a more immediate grasp of the interaction effects detected, one can look at the bar graphs of the three cell types across P,D vs PxD correction conditions given in fig X.3. It is clear from these graphs that the PxD correction affected the locational correlates of HD cells more dramatically than that of TD cells, and left almost untouched those of PC cells. In particular, the locational information (Skaggs and Burgess) decreased when the correction was introduced for both TD and HD cells, suggesting that some of the locational selectivity attributed to these cells was artefactual and that this was particularly true for HD cells.

A similar effect, although in the opposite direction is seen for place field size, which increases for both TD and HD, but in this case the effect is more dramatic for TD cells.

Locational field position

The analysis presented above was also applied to the locational field distribution for the three cell classes. The relative boxplots and bar graphs for field position expressed as a ratio of distance from the wall (see previous chapter, p. 88) are shown in fig X.5. The results were surprising, given that while the main effect of cell type on locational field position reached significance [$F(2,48) = 8.72$, $p = 0.001$], no effect of PxD correction was found [$F(1,48) = 0.778$, $p = 0.382$].

A more interesting effect was obtained for field position change across the two conditions. The absolute distance (in cm) between the locational field peaks in the P,D and PxD conditions was computed for each cell. Boxplots and bar graphs are shown in fig X.5B-C. It is clear that while for almost all the place cells there was no

difference between the position of the peak before and after the correction, TD place peaks shifted somewhat, and the shift was more pronounced for HD cells. An ANOVA confirmed that there was a significant difference in cell type [$F(2,48) = 12.556$, $p < 0.001$], and *post-hoc* tests showed that the shift in locational field position before (P,D) and after (PxD) the correction was significantly higher in HD cells than in both PC and TD cells (NK, $p < 0.05$). The mean amount of locational field shift did not significantly differ in PC and TD cells (NK, $p > 0.05$).

Directional firing properties

The directional variables considered were those listed in the previous chapter: directional selectivity, Skaggs directional information, Burgess directional information and directional range. Table II and III provide a summary of the mean and SEM values obtained for PC, TD and HD cells across the directional variables considered.

The main effect of cell type was significant for all the variables studied: directional firing ratio [$F(2,48) = 12.94$, $p < 0.001$], Skaggs directional information [$F(2,48) = 19.65$, $p < 0.001$], Burgess directional information [$F(2,48) = 27.41$, $p < 0.001$] and directional range [$F(2,48) = 20.02$, $p < 0.001$]. For all variables examined *post-hoc* tests revealed that each cell type differed significantly from each other group (NK, $p<0.05$). Again, the mean values of TD cells were intermediate between those of PC and HD cells across all directional measures tested. This indicates that, as expected, the directionality of PC cells is very modest in comparison to that of HD cells, while TD cells are more directional than PC and less than HD cells.

There was a significant effect of PxD correction for all the variables studied: directional selectivity [$F(1,48) = 31.69$, $p < 0.001$], Skaggs directional information [$F(1,48) = 37.54$, $p < 0.001$], Burgess directional information [$F(1,48) = 12.92$, $p = 0.001$] and directional range [$F(1,48) = 21.62$, $p < 0.001$].

Significant interactions between cell type and PxD correction were found for: Skaggs directional information [$F(2,48) = 3.48$, $p < 0.05$], Burgess directional information [$F(2,48) = 3.56$, $p < 0.05$] and directional range [$F(2,48) = 12.95$, $p < 0.001$] (see fig X.4 for a graphical description of these results). No significant interaction between the two factors was found for directional firing ratio [$F(2,48) = 0.13$, $p = 0.168$].

Summary and discussion

This chapter focused on the relative contributions that location and direction have on topodirectional firing. It was possible to ask this question since, in this chapter, similar number of bins were chosen for the locational maps (70) and the directional plots (64). This allows a fair comparison between the two variables. Additionally, sampling time was increased from 8 to 20 minutes, to improve the reliability of directional and locational sampling.

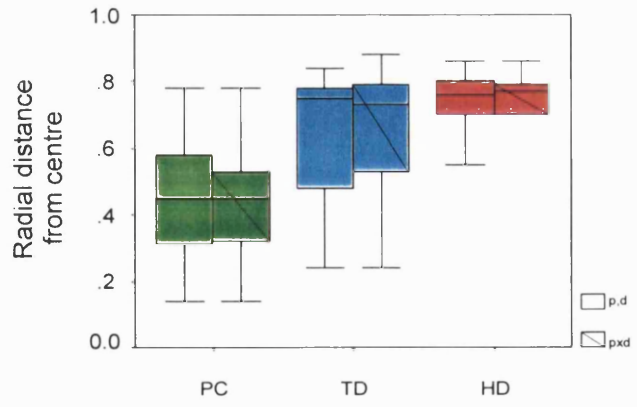
Fig X.5 Characteristics of Locational Peak preferences in place cells (green), topodirectional cells (blue) and head direction (red), before (P,D) and after (PXD) correcting for spatial bias.

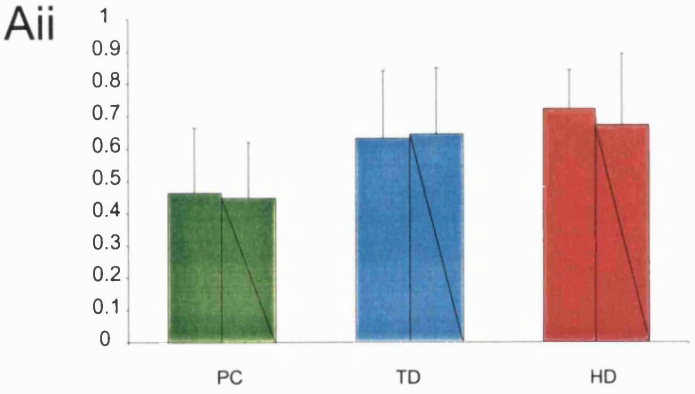
A) Box plot (Ai) and mean and standard error plot (Aii) of Radial distance of Locational Field Peak from Centre of Environment. Within each cell group, left box is uncorrected firing, right box containing diagonal line is corrected for spatial bias by the PXD procedure. Place cell peaks are evenly distributed, topodirectional cell peaks are more skewed towards the cylinder edge, head direction peaks are found almost exclusively along the edge. The PXD correction affects more strongly the position of head direction peaks, shifting the average peak position towards the centre of the cylinder.

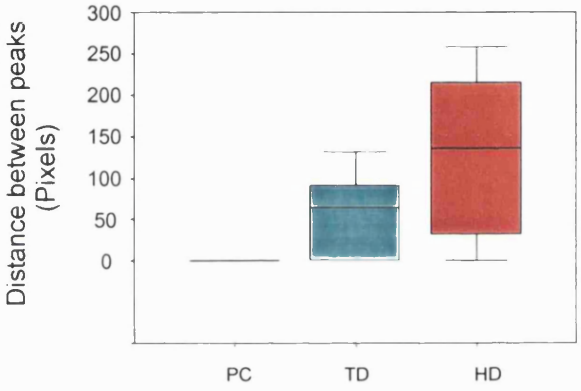
B) Box plot (Bi) and mean and standard error plot (Bii) of the shift in Locational Peak position between the corrected (PXD) and uncorrected (P,D) data. PXD correction leaves mostly unchanged the position of the locational peaks of place cells (green), mildly affects those of topodirectional cells (blue) and severely shifts those of head direction cells (red).

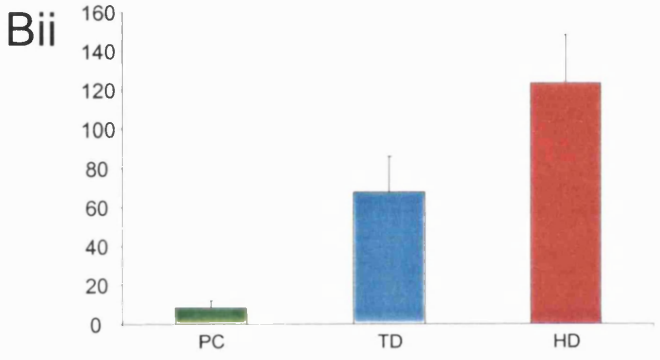
C) Shift in the Locational Field Peak position before and after the PxD correction was applied to place cells (left), topodirectional cells (middle), and head direction cells (red). The coloured dots represent the peak position for each cell in the cylinder, the arrow points to the new peak position after PXD correction was applied.

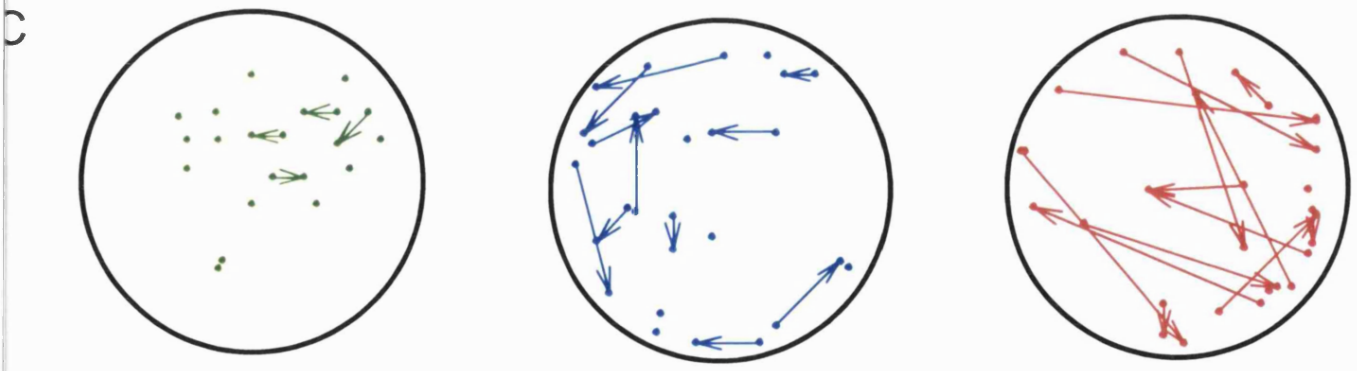
Applying the PXD correction procedure, mainly shifts the position of head direction peaks, suggesting that they are mostly artefactual. Topodirectional peaks are mildly affected by the correction, and place cell peaks stay mostly unchanged.


Radial distance from centre
1.0
.8
.6
.4
.2
0.0
p.d
pxd
PC
TD
HD


Aii
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
PC
TD
HD


Bi
Distance between peaks (Pixels)
300
250
200
150
100
50
0
PC
TD
HD


Bii
160
140
120
100
80
60
40
20
0
PC
TD
HD


C

The results obtained from the distributive hypothesis procedure have confirmed that TD firing is modulated by both location and direction, by showing that neither of these variables on its own can predict TD firing patterns in a satisfactory way. This differs to that shown for PC and HD cells. PC cells' locational firing was successfully used here to predict their directional responses, closely replicating Muller et al (1994) results. Moreover, the locational firing of HD cells was almost perfectly predicted by their directional firing, indicating that the locational responses of HD cells are mostly artefactual. Thus, it was confirmed that the primary correlate of PC cells' firing is location, that of HD cells' direction, and shown that both the animal's location and head direction are determinants of TD cells' firing.

In addition to this, it appeared that for TD cells, the average value of the directional distributive ratio is larger than that of the locational distributive ratio. This indicates that TD directional firing is less predictable on the basis of locational firing than vice versa, suggesting that TD cells might receive stronger directional than locational inputs.

In the second half of the chapter a new algorithm (PxD) was presented and used to correct for the effects of behavioural biases on both the locational firing maps and directional plots. The firing characteristics of TD cells were then compared to those of PC and HD cells, in both the uncorrected (P,D) and corrected (PxD) versions. The firing characteristics of these three cell types was assessed through and compared across a variety of directional and locational firing measures. These were: locational/directional selectivity, locational/directional information measures (Skaggs and Burgess), spatial coherence (for locational maps), locational field size, and directional range.

TD values for each of these variables were always found to be intermediate between those of PC and HD cells (in both the uncorrected and corrected versions). In particular, as expected, PC cells had the largest average values on all the locational firing measures considered, HD cells the lowest, and TD cells had intermediate values, somewhat closer to those of HD cells. A very similar, albeit inverted, pattern was seen across the directional measures: HD cells had the largest average values on all the directional measures used, PC cells the lowest, and TD cells had intermediate values, again, more similar to those of HD cells.

These results confirm and extend those obtained through the distributive hypothesis procedures: the firing of TD cells is modulated by both the animal's location and head direction. Locational and directional firing of TD cells is robust, given that the PxD correction does not eliminate the contribution of either variable to the cells' firing, while severely decreasing the contribution of head direction on PC cells' firing (see fig X.4) and of location on HD firing (see fig X.3).

The fact that on virtually all measures of spatial selectivity used TD values were always closer to those of HD cells indicates that the firing of TD cells is more deeply modulated by head direction than by location. This is also confirmed by directly comparing the average values of locational and directional information measures for TD cells. The directional information values are larger than the locational ones, for both the uncorrected and corrected data (P,D and PxD). It thus appears that, on average, TD cells carry more directional than locational information.

It is unlikely that this result is artefactual, given that there were more locational (70) than directional (64) bins, and this should, if anything, increase the values of locational information over those of directional information. Having a closer look at the data, it emerges that TD cells could be separated into two sub-populations: 70% of the cells show higher directional than locational information, while for the remaining 30% of the cells the opposite is true. Examples of cells for which location is the primary firing correlate are: TD a25, a28, f3, f5 and f6 (see fig IX.3). Examples of TD cells for which head direction is the primary firing correlate were: TD a17, a27, b5, d2, f4.

TD cells might thus represent a heterogeneous population, with the majority of them receiving stronger directional than locational inputs.

In conclusion, these results provide evidence that TD cells firing is the result of both the animal's location and head direction, with the majority of TD cells having a stronger directional than locational modulation.
Locational and directional firing of TD cells is robust, though TD cells carry less locational information than PC cells and less directional information than HD cells.

Cells whose firing is correlated to, in different proportion, the animal's location and head direction have been found throughout the hippocampal formation. In general these cells are modulated by only one of these two variables. At the two extremes lie the hippocampal place cells, whose firing is determined almost exclusively by location, and the presubicular head direction cells, whose firing is determined by head direction. Other, more hybrid, firing patterns have been described. Location was found to be the main correlate for entorhinal (Quirk et al, 1992, Frank et al, 2000 and 2001), subicular (Sharp and Green, 1994) and parasubicular (Taube, 1995c) neurons. Firing of medial and lateral entorhinal neurons seems to be exclusively influenced by the rat's location, even though entorhinal neurons carry less locational information than hippocampal place cells.
Subicular neurons show a secondary directional component to their firing, although direction was described as having a "reliable but very small influence on firing rate" (Sharp and Green, 1994).

Only three (out of 18) parasubicular "place" cells had a secondary directional component to their firing (Taube, 1995c), for the remaining 15 cells no significant influence of head direction on firing rate was detected. Theta modulation of these cells' firing was not tested. Therefore some or all of these 3 cells might have potentially been very similar to TD cells.

Another cell population which was found to reliably combine locational and directional responses was isolated from the presubiculum by Sharp et al (1996). As already anticipated, only two out of 14 cells included in this place-by-direction category showed theta modulation, the other hallmark of TD cell firing.
In summary, cells whose firing is influenced by both location *and* head direction have been described in both the pre- and parasubiculum. Theta modulation was tested and positively attributed to only a subset of these cells.

It remains very difficult to compare directly the properties of cells recorded in different laboratories. This issue is further complicated by the fact that different

analytical methods were used to describe the spatial properties of the cells in question. In the case of the study by Sharp et al (1996), for example, cells were classified using a partial correlation method, which has not been used in this thesis.
Therefore, it is impossible to strictly compare the properties of the cells recorded by Sharp et al (1996) and Taube (1995c) to the TD cells presented in this thesis.

In conclusion, TD cells might be viewed as belonging to the broad family of place-by-direction cells described in the pre- and parasubicular cortices (Taube, 1995; Sharp et al, 1996), with the addition that their firing is, in almost all the cases studied, strongly modulated by theta (this might have interesting functional implications).

The possible anatomical origin of TD cells' locational and directional signals and their functional significance will be discussed in detail in the general discussion section of this thesis.

Chapter XI

Directional stability and directional constancy of topodirectional cell firing

This chapter focuses on how environmental changes affect the directional properties of TD cell firing.
In particular, two issues will be discussed: the stability of the TD preferred direction across recording sessions conducted in similar environments, and the constancy of TD directional signal within and outside the curtained environment.

Directional stability

The activity of 34 TD cells was recorded during two 8-minute trials, in the cylinder, inside the curtained enclosure (see fig VI.2 in methods section). A variable number of trials intervened between the recording sessions considered in this analysis. Before recording began, the animals were brought inside the curtained environment in a standard way, always facing north, and were placed in the middle of the cylindrical environment.
Directional plots contain 36 bins, no smoothing is applied.
The angular difference between the preferred directions of each TD cell in the two trials was computed. Fig XI.1 shows the distribution of the differences between preferred direction across the two trials for the 34 cells. The average difference was – 0.29° (±3.76). A pairwise two-tailed t-test revealed that the preferred directions across the two trials were not significantly different ($df = 33$, $t = 0.078$, $p = 0.94$).
This result shows that TD directional firing is stable across recording trials conducted in the same environment. This result is similar to what is found in HD cells (Taube, 1998).

Directional constancy

The experiment described below was designed to test whether the directionality of TD cells is constant within and outside the curtained enclosure. Given that these two environments share very few sensory features, this is a way to assess if the directional signal seen in TD cells is allocentric (similar to HD cells), or if it is rather tuned to sensory features specific for each environment (very much like local view cells).

The activity of 15 TD and 15 HD cells was monitored under two different conditions, while rats ran:

1) in the cylinder inside the curtained enclosure;
2) on the holding platform, outside the curtained environment.

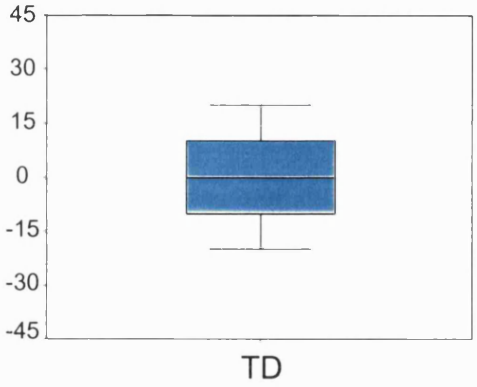


Fig XI.1 Boxplot of difference between the preferred directions of topodirectional cells over two trials. The average difference was -0.29 degrees, showing that directional firing of topodirectional cells is stable across recording sessions.

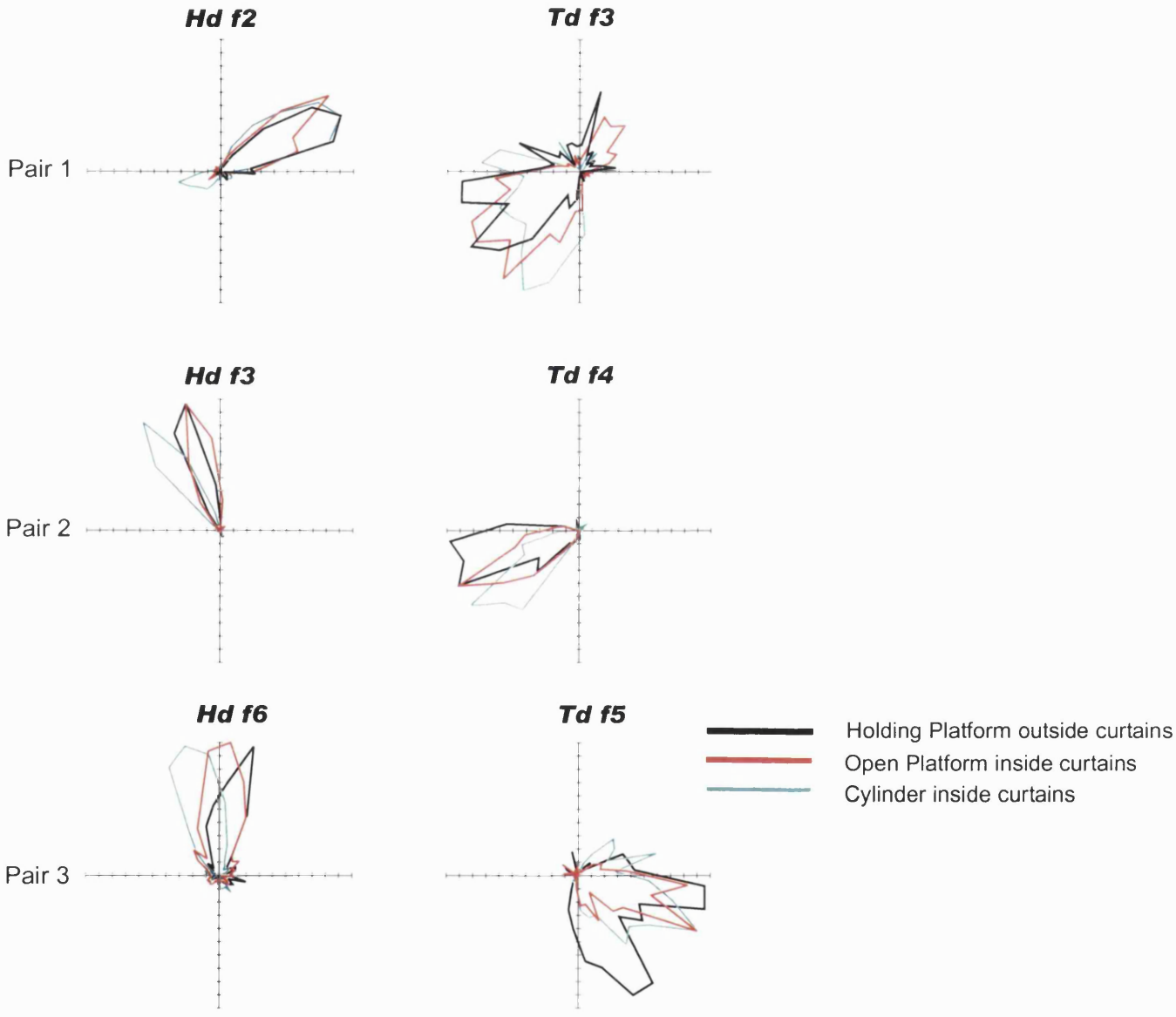


Fig XI.2 Uncorrected (P,D) directional plots of three pairs of simultaneously recorded head direction (left) and topodirectional (right) cells. Cells were recorded in three environments: holding platform (black outline), open platform (red), cylinder (blue).
Directional profiles across the three environments are generally similar, for both head direction and topodirectional cells. Where differences can be seen (pair 2) the directional tuning of the cells rotate in unison (counterclockwise rotation in cylinder for both head direction and topodirectional cell).

The difference between the preferred directions in conditions 1 (cylinder) and 2 (holding platform) was computed for each TD and HD cell recorded.
Additionally, the activity of 9 of the 15 TD cells was recorded while rats ran:

3) on an unbounded circular platform inside the curtained environment.

The difference between the preferred directions in conditions 3) (unbounded platform) and 2) (holding platform) was calculated for these 9 TD cells.
The animals were brought in and out of the environments using standard procedures (see Methods section p. 65), so that they were never purposefully disoriented.

Fig XI.2 shows the uncorrected directional polar plots for three pairs of TD and HD cells simultaneously recorded in the three conditions. The directional profiles for each condition are superimposed: blue indicates cylinder, black holding platform and red unbounded circular platform. It is clear that the preferred directions are constant across conditions, and when differences are found (see the cylinder trials for pair 2), they are the same for the HD and TD cell. This suggests that under these conditions the directional signals coming from TD and HD cells change in unison.

Preferred direction values were computed for both the uncorrected (P,D) and the corrected (PxD) data. The number of directional bins used was 36 and no smoothing was applied to the directional maps.
Pairwise two-tailed t-tests were conducted to verify if there were significant differences in the preferred directions of cells recorded across the conditions described above. Table IV summarises the results obtained.
First of all, the preferred directions of HD cells did not change significantly when cells were recorded in either the cylinder or the holding platform. This was found to be true for both the uncorrected (P,D; mean difference = 4°, $t = 1.10$, $p > 0.1$, $n = 15$) and corrected (PxD; mean difference = -1.43°, $t = -0.31$, $p > 0.1$, $n = 14$) maps, confirming the likelihood that HD cells are coding direction in an allocentric framework. It was possible to obtain corrected values of the preferred direction across the two environments only for 14 out of the 15 cells originally considered. In one case the PxD algorithm did not converge, and no solution was found.

In the case of TD cells, there was a significant difference in the preferred directions of cells recorded in the cylinder compared to the holding platform conditions, but only if the data was not corrected for sampling biases (P,D; mean difference between preferred directions = 23.33°, $t = 3.29$, $p < 0.01$, $n = 15$).

After applying the PxD correction (PxD), no significant difference was detectable in the preferred directions (mean = 8.57°, $t = 1.41$, $p > 0.1$, $n = 14$). This suggests that sampling bias was artificially introducing a difference between the preferred directions of TD cells recorded in the cylinder and the holding platform. This is probably because while the holding platform is an unbounded environment, the cylinder has walls and these pose special kinds of behavioural constraints on the sampling of head directions (see previous chapter). To test this hypothesis 9 out of the 15 TD cells were tested under condition number 3) (unbounded circular platform inside the curtained enclosure). The results were that no significant difference was found between the preferred directions of TD cells between these two unbounded environments for both the uncorrected (P,D; mean = 0.00°, $t = 0.00$, $p > 0.1$, $n = 9$)

and corrected (PxD, mean = 2.5°, t = 0.48, p > 0.1, n = 8) data. This suggests that the difference originally found in the uncorrected preferred directions of TD cells in the cylinder compared to the holding platform may be due to the presence of walls in the cylinder.

In summary the data presented shows that the preferred directions of TD cells do not vary across environments which share very few (if any) perceptual features. This suggests that TD cells do not act like local view cells, but that they code for head direction in an allocentric framework, similarly to HD cells. (It also suggests that idiothetic cues alone can maintain a stable directional signal in TD cells, at least for the brief time required to move the rat across the curtains).

Chapter XII

Theta modulation of topodirectional cell firing

Topodirectional cell firing shows a marked rhythmicity at theta frequency (9-10 Hz).
This chapter focuses on two related issues: the degree of theta modulation of TD firing, in comparison to that found in PC and HD cells, and the way theta phase affects the firing probability of TD cells.

Methods

Theta phase was determined by fitting the EEG data to a sequence of sinusoids (see Methods section, chapter VI, p.69). Only data points for which this fitting was possible were considered. There is great variability in the quality of the theta oscillation recorded from the brain areas considered (see fig. XII.1), consequently the proportion of spikes to which a theta phase could be assigned varied across cells. Only cells for which at least 50% of their spikes were assigned a theta phase are included in the analysis.

Cell samples

Cell samples included 17 PC, 30 TD and 14 HD cells.
TD cells were recorded from 7 rats (rat a = 15 cells, b = 5, c = 1, d = 1, e =1, f = 6, g = 1).
HD cells were recorded from 5 rats (rat a = 1 cells, f = 4, i = 1, k = 4, n = 4).
In some animals both TD and HD cells were recorded (rats a and f). TD and HD cells were simultaneously recorded on two occasions: HDf1-2 and TD f3 in one instance, HDf6 and TDf5-6 in the other.

PC cells were recorded by Colin Lever, from CA1, in one rat (rat o).

Table IV								
	TD cells						**HD cells**	
	p,d	pxd		p,d	pxd		p,d	pxd
	cyl-hp	cyl-hp		open pl-hp	open pl-hp		cyl-hp	cyl-hp
	(deg)	(deg)		(deg)	(deg)		(deg)	(deg)
number of cells	15	14*		9	8*		15	14*
mean	23.33	8.57		0.00	2.50		4.00	-1.43
sem	7.08	6.10		7.82	5.26		3.63	4.67
df	14.00	13.00		8.00	7.00		14.00	13.00
t	3.29	1.41		0.00	0.48		1.10	-0.31
sig.	p<0.01	p>0.1		p>0.1	p>0.1		p>0.1	p>0.1
*in one case the PXD algorithm did not converge								

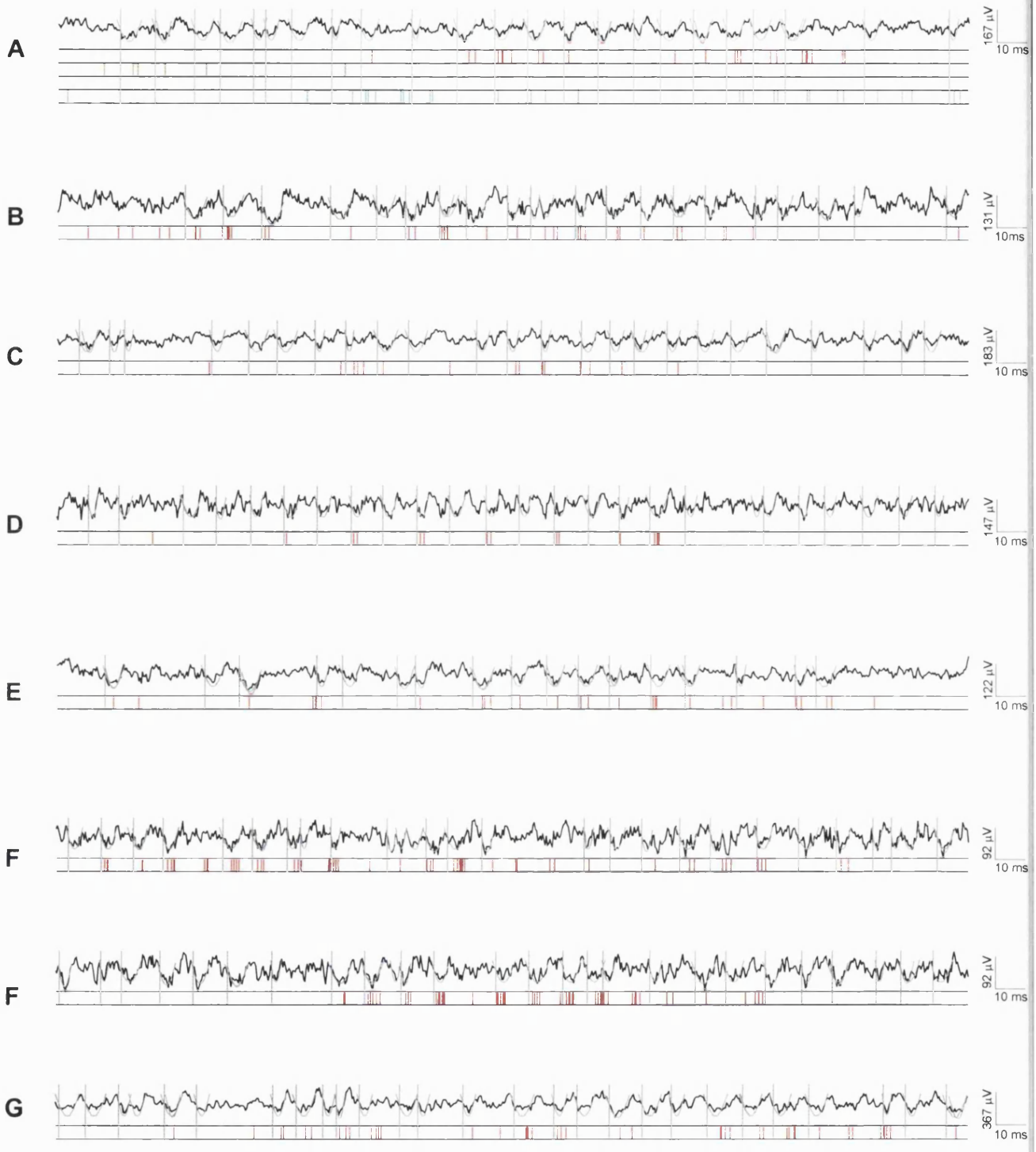


Fig XII.1 Examples of topodirectional cell firing in relation to the theta EEG oscillation from each of the seven animals from which topodirectional cells were recorded.

For each animal from A to G (left of trace: same letter identification as elsewhere in thesis) 3 seconds of representative data are shown, the EEG trace on top, spike occurrence on the bottom. The analysis software assigns theta wave inflections to the raw EEG trace data. Grey valley-marks show where the software has assigned the theta trough; grey vertical lines denote the 0/360 degree point in the theta cycle.

It can be seen the topodirectional cell firing is highly modulated by the theta oscillation, spikes tending to occur at the trough of the theta wave.

For each cell, the total number of spikes derived from several recording sessions was used. This was possible because no difference was found in either theta modulation or theta preference across trials.

Results

Fig XII.1 shows 8 representative 3 seconds EEG traces derived from each of the 7 rats included in this study (two traces for rat f are shown).
The light grey curved lines indicate which portions of the EEG traces were fitted to half-sinusoids, and the grey vertical lines specify phase zero of the theta oscillation.

Vertical colour lines indicate spikes occurrence, A refers to a recording session where three TD cells were simultaneously recorded, in each of the other cases only one TD cell was recorded. We can see that TD spikes a) have a quite deep modulation by theta and b) occur around the trough of the locally recorded theta oscillation. We consider a) first.

Degree of modulation by theta

All the topodirectional cells recorded (N = 46) showed a modulation at theta frequency. Fig XII.2 shows the autocorrelograms for the entire dataset of this thesis, taken from single trials in the cylinder. We can see purely by visual inspection that virtually all the cells are moderately to highly modulated at the theta frequency (around 9 Hz). There are few exceptions to this. Note that TDa10 and TDc1 are mainly modulated at half this frequency while cells TDb5, TDd1 are only mildly modulated and TDe3 shows an almost undetectable modulation in the autocollelogram shown.

In order to assess more quantitatively the depth of theta modulation of TD cells firing, several trials were combined for each cell, to increase the number of spikes included in the analysis. A theta phase value was assigned to each spike which occurred during EEG epochs that could be fitted (at least 50% of the spikes the cell fired during the trials considered). Theta phase distributions for each cell were tested for uniformity, using the Rayleigh test. All TD and PC cells passed the test, indicating that spikes did not occur randomly at all theta phases ($p < 0.001$). Not surprisingly none of the 14 HD cells passed the test. HD spikes occurred at all theta phases, showing that HD firing is not modulated by theta.

Examples of theta phase distributions for the three cell types are shown as circular histograms in fig XII.3. TD and PC cells theta phase distributions can be modelled as von Mises distributions, the equivalent of the normal distribution for circular data. The mean phase angle and concentration parameter were computed for each cell.

We take the concentration measure as an index of the depth of theta modulation. This is because it reflects the dispersion of the data (being analogous to the variance in linear statistics), and thus indicates the degree of rhythmicity for each cell. High concentration values, imply that the data are "peaked" around a particular

theta phase value, indicating that cell firing is strongly modulated by theta, low concentration values indicate that theta modulation is quite loose.

Concentration values are shown for some of the autocorrelograms in fig XII.2, and all the circular histograms in fig XII.3. There is good agreement between the depth of theta modulation that one intuitively assigns through visual inspection to the autocorrelograms and concentration values computed for the underlying theta phase distributions. This suggests that the concentration measure is a good estimate for the depth of theta modulation. From fig XII.3 it is clear that concentration values for TD cells are higher than those of PC cells, while HD cells values are very low.
This is confirmed by the boxplots and bar graphs shown in fig.XII.4, which depict the distributions of the concentration measures across the three cell populations.

Concentration does not depend on the number of spikes fired ($r = 0.004$, $p = 0.976$), ruling out that the uneven number of spikes across cell type could contribute to the difference in concentration seen.

A one-way ANOVA confirmed that the main effect of cell type on concentration was significant [$F(2,58) = 56.10$, $p < 0.001$], and Scheffe's *post-hoc* tests revealed that each cell group was significantly different from each other ($p < 0.01$). In particular, TD cells had the highest average value of concentration (0.73 ± 0.04), followed by PC cells (0.39 ± 0.04) and HD cells (0.10 ± 0.02).

In fig XII.5 concentration values for PC, TD and HD cells included in this study are shown together with those found in septal cells by King et al (1998). The degree of theta modulation of TD cells appears to be very similar to that of type Ia septal cells, and only type Ib cells show a deeper theta modulation than TD cells.
In conclusion, TD cell firing is highly modulated by theta, more so than PC firing, while HD cells show very little (if any) theta modulation.

Theta phase preference

We have already seen examples of TD firing suggesting that spikes occur around the trough of the locally recorded theta oscillation (around 90 degrees, in the theta phase histograms). We can now plot the mean values of theta phase for all PC, TD and HD cells. Results are summarised in fig XII.6A and C, as circular histograms and boxplots, respectively.
A value of 90° is conventionally assigned to the trough of the theta oscillation and of 270° to its peak.

TD mean phase values are highly clustered around a mean of 79.50° (± 6.17), PC values are less tightly clustered around a mean of 193.92° degrees (±14.41), and HD cells mean theta phase distribution is highly dispersed, with a mean of 47.92° (± 94.53). Only TD and PC distributions are significantly clustered around their mean values (Rayleigh tests, $p < 0.001$). These results indicate that TD cell firing preferentially occurs just before the trough of the locally recorded theta oscillation, very similarly to what is reported by Csicsvari et al (1999) for interneurons in CA1 stratum pyramidale. Note also that mean theta phase values of cells recorded from different animals are very similar, and that the variability of phase preference within

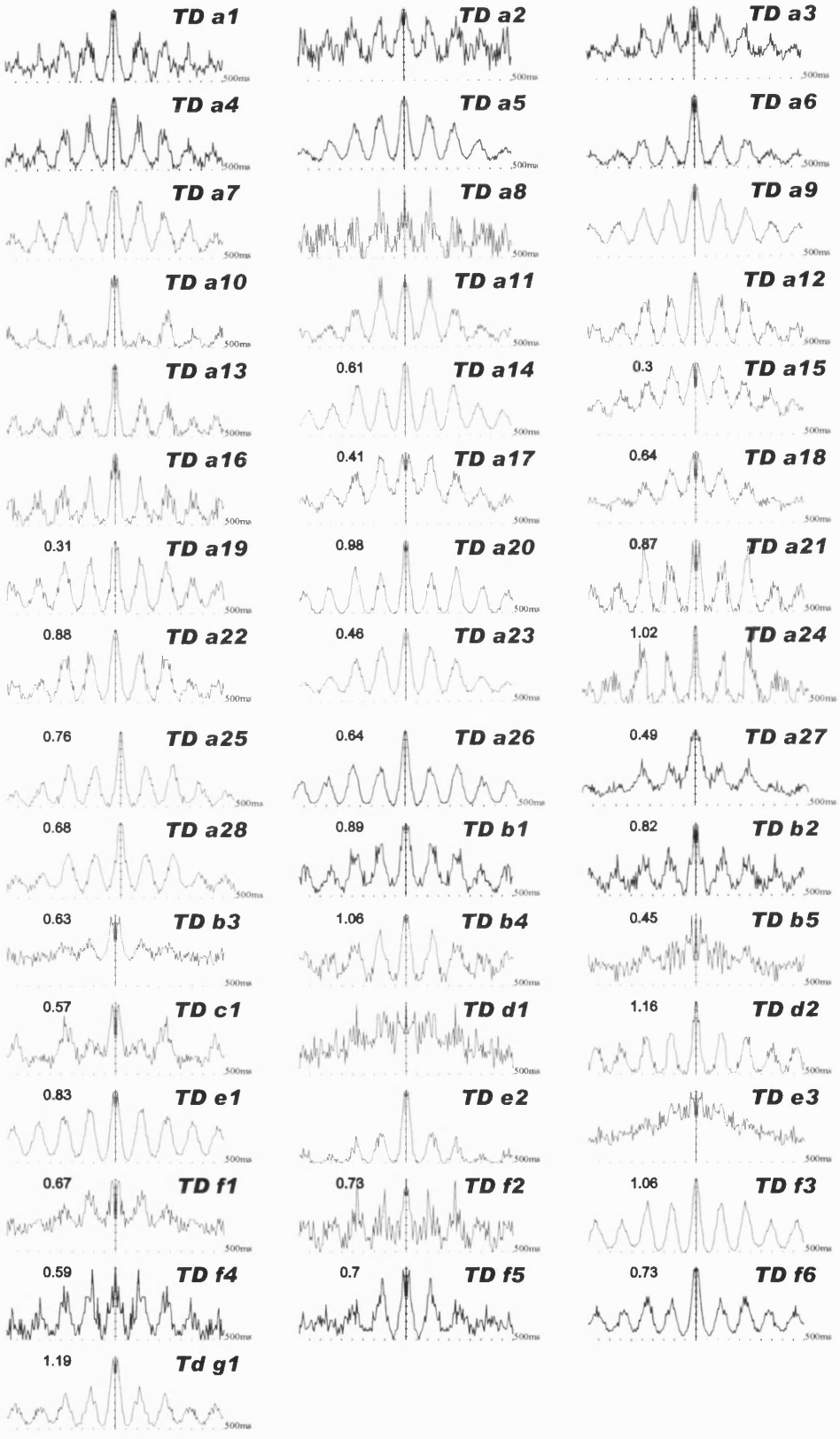
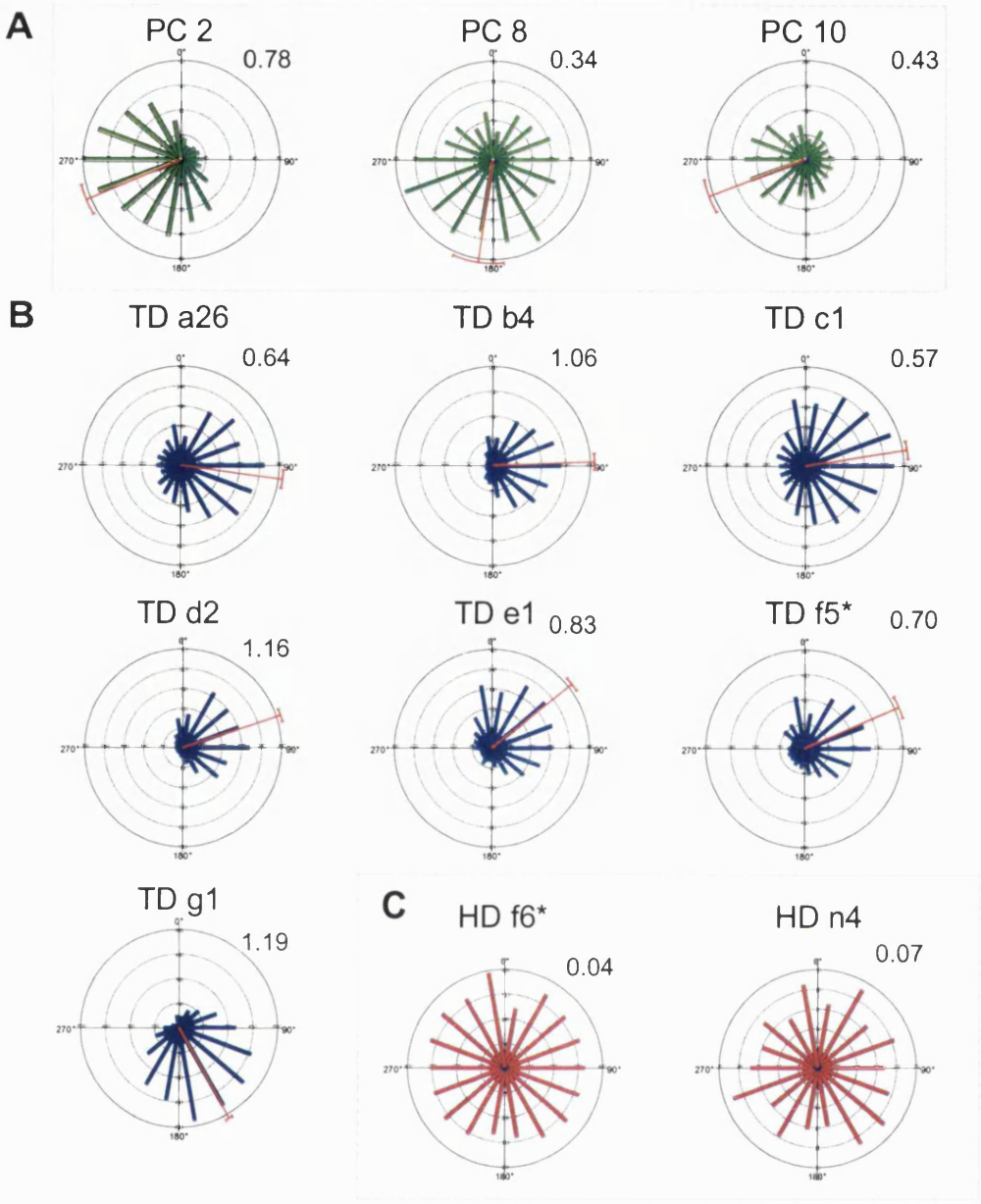


Fig XII.2 Autocorrelation histograms for 500ms period for each of the topodirectional cells recorded. Data from canonical trial in cylinder are shown.

Concentration values are shown top left of each histogram, where this has been calculated . The timing of each spike relative to the phase of local EEG theta is derived; concentration measures the circular variance of a given cell's spikes around the mean phase of all its spikes, and is used here as a measure of rhythmicity.

Virtually all the cells are moderately to highly modulated by the theta oscillation.



g XII.3 Phase preference histograms showing firing of three cell types relative to theta phase.

ach cell spike is assigned to a phase (0-360 degrees) of local theta; each histogram shows spike uantity in each phase in 20 degree bins. Arbitrarily, 90 degrees (3 o'clock) represents the trough, and 70 degrees (9 o' clock) the peak, of the local theta wave. Concentration values are shown top right of ach histogram. The range of the 95 percent confidence interval is shown in red.

epresentative examples of place cells (A), topodirectional cells (B), and head direction cells (C) are nown. One topodirectional cell is shown for each animal from animal A to animal G in letter order.

ne head direction cells do not fire at any particular phase of theta, and show low concentration scores. ne place cells are moderately influenced by theta, firing in the 90-180 degree quadrant, showing oderate concentration scores. The topodirectional cells are highly influenced by the theta oscillation, ing in and around the 90 degree region, and show moderate to high concentration scores.

Note that cell TD f5 (concentration = 0.70) and HD f6 (concentration = 0.04) were recorded multaneously, showing that the demonstrated lack of theta rhythmicity in the head direction cell cannot e explained by some kind of incoherent yet theta-esque EEG signal.

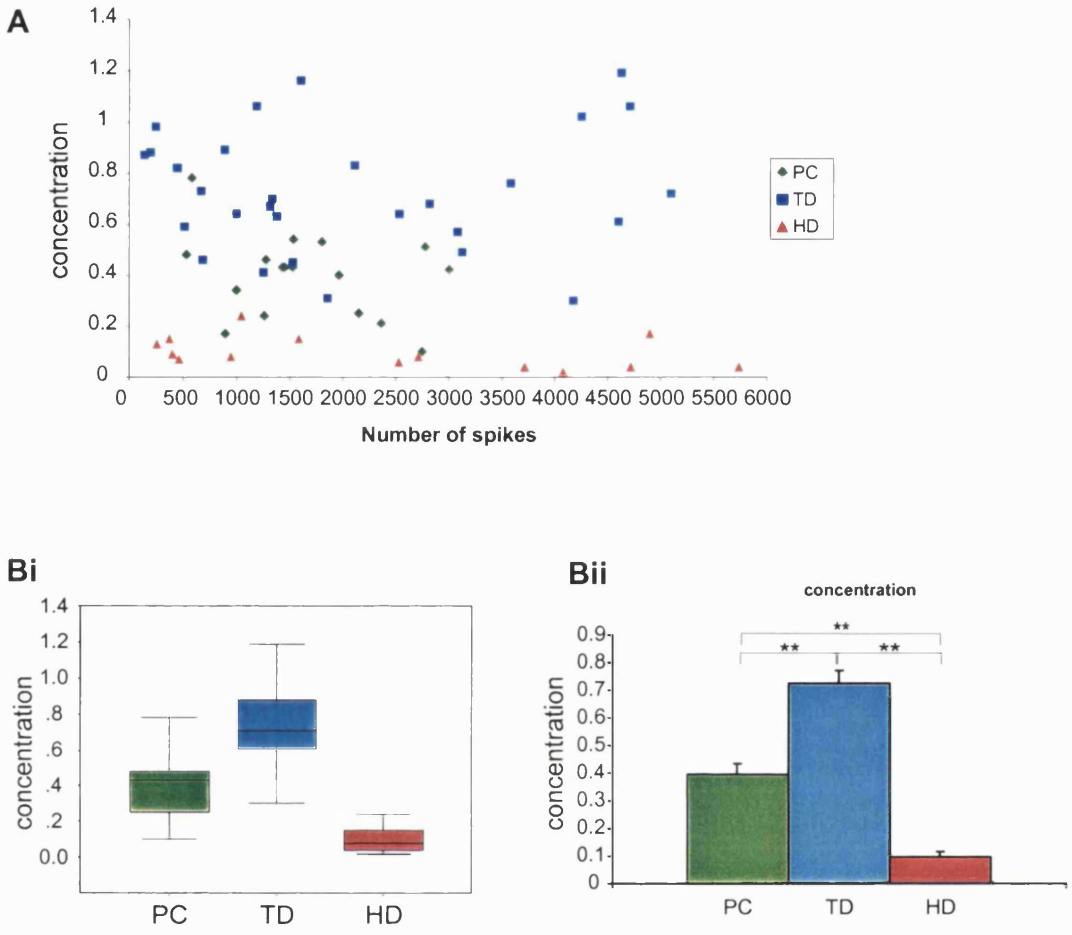


Fig XII.4 The degree of rhythmicity in the three groups of cells.

"Concentration" measures the circular variance of a given cell's spikes around the mean phase of all its spikes. Low concentration means that spikes tend to occur at all phases of the theta oscillation; high concentration means that the spikes tend to be clustered at a particular phase.

A) Scatter plot showing that concentration is not correlated with spike quantity in the cell groups.
B) Descriptive summary of distribution of concentration values in the three cell groups.
Bi) Box plot, and Bii) Mean and standard error plot, of concentration values

It can be seen that place cells show moderate rhythmicity, and that topodiirectional cells show quite high rhythmicity.

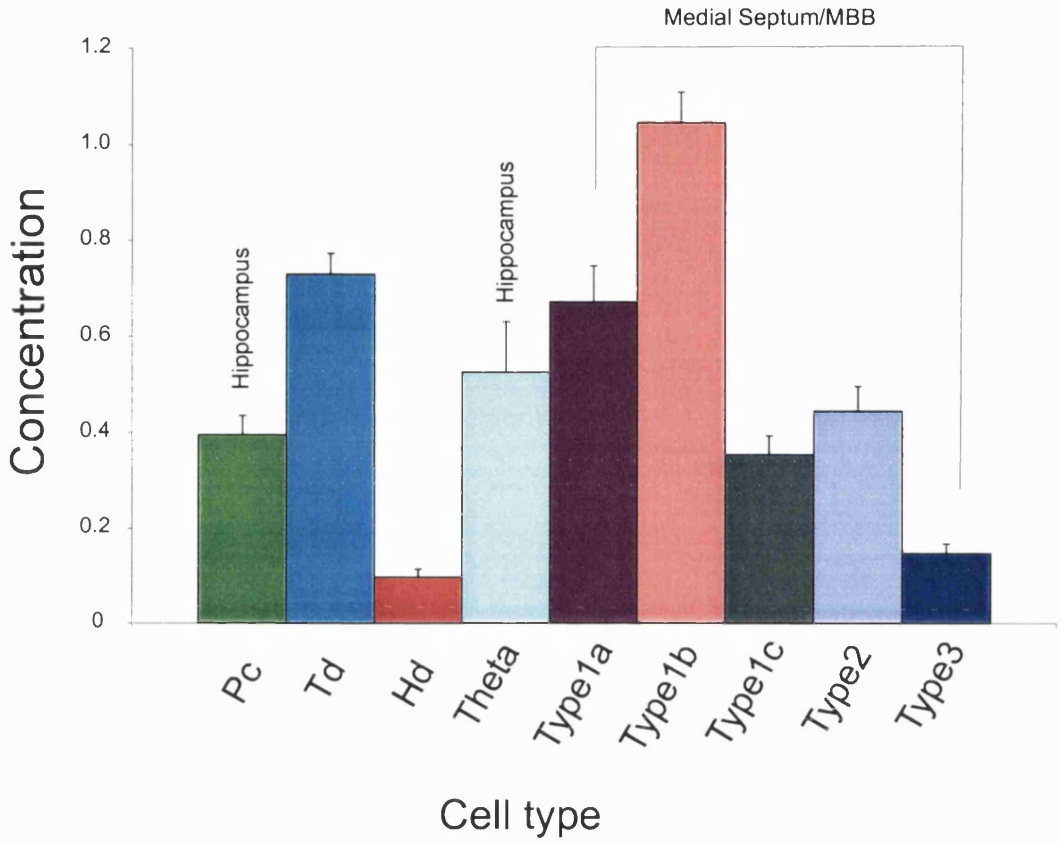


Fig XII.5 Theta rhythmicity, as measured by theta phase concentration, in a variety of cell types in the septo-hippocampal system.

Bar Chart shows mean and standard error of concentration for each cell type.

Abbreviations and information about each cell type:
Pc = Place cell sample from this thesis, recorded from CA1 pyramidal layer of the hippocampus. Theta was recorded from this region.

Td = Topodirectional sample from this thesis, recorded as described.

Hd = Head direction cell sample from this thesis, recorded as described.

Theta = Interneurons sample from this thesis, recorded in and around the CA1 pyramidal layer of the hippocampus. This is a heterogenous sample characterised by waveform shape and high mean firing rate, with no exclusion of any cells.

Type 1a-c, 2, and 3 = Cells in the medial septum and diagonal band of Broca (MS/DBB), taken from and characterised according to the study of King et al (1998). Cell spikes were referenced to theta in the hippocampal fissure.

Note that the topodirectional cells show higher concentration than the hippocampal interneurons, and than all the MS/DBB cell categories except type 1B.

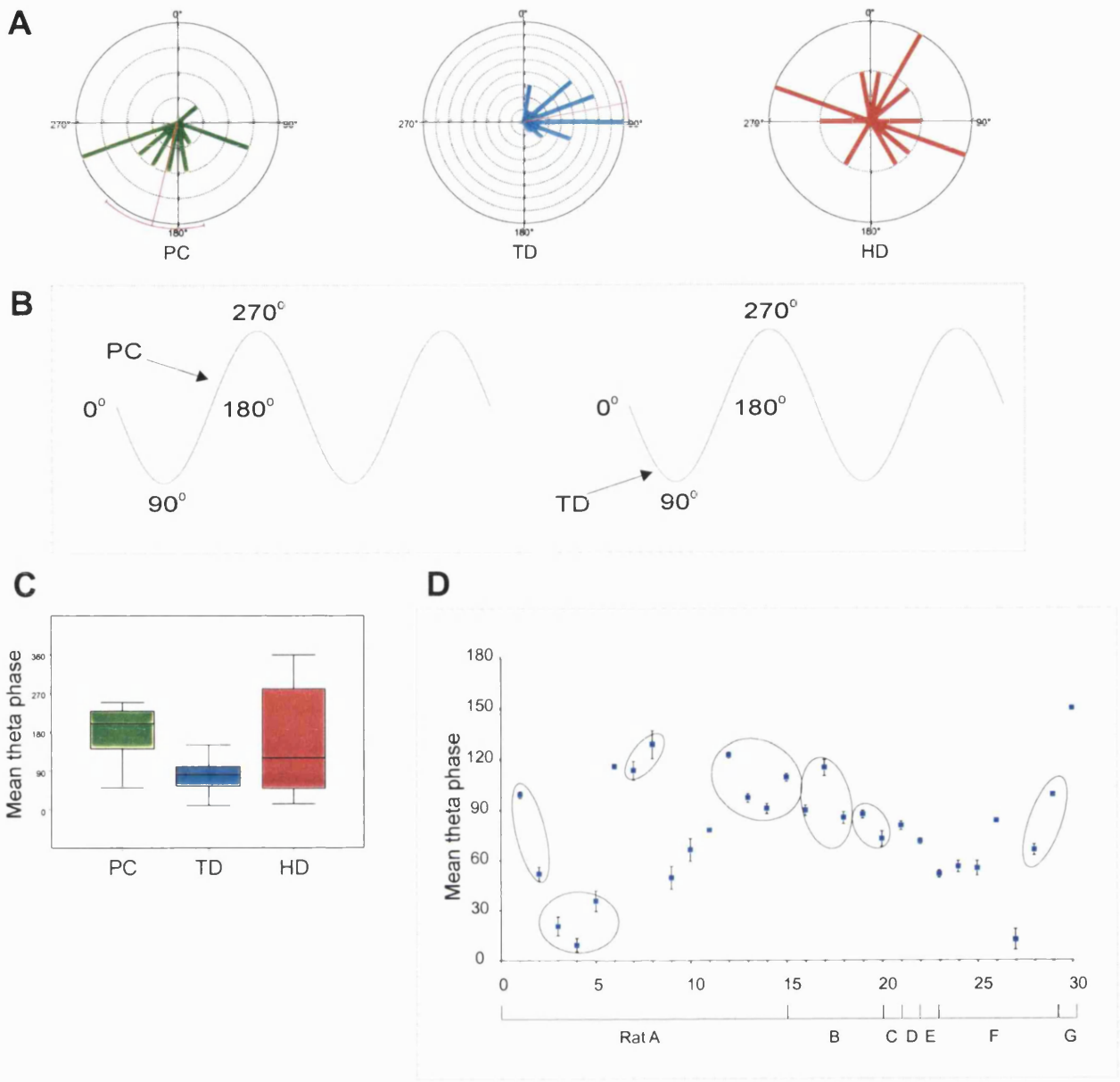


Fig XII.6 Mean theta phase angle of the three cell types.

Here, the mean phase angle is derived for each cell, so that each cell contributes only one phase value to the sample. This mean angle is calculated irrespective of whether the concentration around this mean is statistically significant. The question now considered is:"does each cell type tend to show a particular phase preference that is consistent across the members of that cell group?". These figures illustrate that the answer to this is "Yes" for the place and topodirectional cells, and "No" for the head direction cells.

A) Mean theta phase histograms for place cells (left), topodirectional cells (middle), and head direction cells (right) are shown. Conventions as in previous figure.
B) Schematic cartoon of results of analysis shown in A: yes, place cells and topodirectional cells tend to fire consistently as a population, showing a mean mean phase of around 200 degrees and 80 degrees respectively. *Note that no direct comparison between the phase preference of TD and PC cells is possible; no common EEG reference was recorded.*
C) Box plot of mean theta phase for each cell group. Note the small variability of the topodirectional cell sample, and the large variability of the head direction sample.
D) Mean and standard error plot of mean phase for each of the topodirectional cells contributing to the analysis of mean phase, arranged in order according to animal. Within each animal, left (early) to right (late) order reflects recording sequence; circled cells denote two or more simultaneously recorded cells. Note the relatively small variability in the sample. No obvious trends relating recording sequence to mean phase angle can be seen from animals A, B, and F.

simultaneously recorded cells does not seem to be different from that between cells recorded during different days or even animals (see fig XII.6D).
Fig XII.6B provides a cartoon of when, in the theta oscillation, TD and PC firing probability is maximal.

Note that no direct comparison between the phase preference of TD and PC cells is possible, given that these values refer to the locally recorded theta oscillations, and it is more than likely that theta recorded from such distant brain areas is not synchronised. Only one TD cell was recorded from just below the CA1 stratum pyramidale (TDg1, see fig XII.3), interestingly, the mean phase preference for this cell with respect to the hippocampal theta was 150.37 degrees (±1.08). This value is significantly different from the TD cells theta phase mean, indicating how critical the choice of EEG reference is.

The only meaningful comparison to be drawn from the results presented, is between the dispersions of the TD and PC cell populations.
PC phase preference is more variable than TD. This possibly reflects the fact that PC phase preference is not fixed, but shifts at earlier theta phases during single traversals through the place field (phase shift phenomenon, see introduction). Although only a small range of data was inspected, no evidence of phase shift was observed for TD cells (but note that it is more difficult to see phase shift in the open field). The rather small range of theta phase values for individual TD cells (see fig XII.3) further suggests that any degree of phase shift must be relatively minor if it occurs.

Discussion

We have shown that: the firing of TD cells is strongly modulated by theta, and that the highest probability of TD firing occurs just before the trough of the locally recorded theta oscillation. No theta modulation was ever observed in HD cells, not even when both HD and TD cells were simultaneously recorded (see fig XII.3).

TD cells are not the only theta modulated cells found in pre- and parasubiculum. In several instances, we have recorded cells with a marked theta rhythmicity in these brain structures. In general, no clear spatial correlate could be found for these cells. An exception was one cell recorded from animal A, which displayed a very deep theta modulation and its firing rate was positively correlated with the animal's running speed (Lever et al, 2003).
No quantitative assessment of the proportion of theta modulated units in pre- and parasubiculum was performed.
Other authors have shown that theta modulated cells represent between 15% (Taube et al, 1990a) and 17% (Sharp, 1996) of the total number of cells recorded from the presubiculum. Taube (1995c) has reported that in the parasubiculum, a higher proportion of cells (41.1%) is theta modulated.

The most likely source of the theta rhythmicity observed in pre- and parasubiculum is the septal formation. Presubiculum receives strong projections from the medial septal nucleus and the vertical limb of the diagonal band of Broca, which terminate mainly in presubicular layer II (Van Groen and Wyss, 1990a, b). Septal

inputs to parasubiculum are very similar to those of presubiculum (Amaral and Witter, 1995). The depth of theta modulation observed in TD cells is similar to that of type Ia septal cells, and lower only to that of type Ib septal cells (see fig XII.5). TD theta modulation is thus comparable in magnitude to that of septal cells which drive theta in the hippocampus (see chapter II, p. 18-20).

Another possible source of the theta modulated firing seen in the presubiculum is the anteroventral nucleus of the thalamus (AV). It has been shown that up to 75% of the cells recorded from the AV fire rhythmically with theta, and 46% of these show a very deep theta modulation (Vertes et al, 2001). It has also been shown that the ventral and the medial quadrants of the midrostrocaudal portion of the AV project, respectively, to layers I and III and to layers I and IV-VI of the dorsal presubiculum (Shibata, 1993).

Thus, while septal inputs selectively target neurons in the superficial layers of pre- and parasubiculum, the AV might directly channel theta modulated firing to the deep layers of presubiculum.

It is interesting that, in some animals, TD cells were recorded from the deep layers of presubiculum (all the TD cells recorded from animal E and the majority of those recorded from animal F, see chapter VII, p.78-79).
Chrobak and Buzsaki (1994) studied the electrophysiological properties of neurons in both the superficial and deep layers of a variety of retrohippocampal areas (subiculum, pre- and parasubiculum, and entorhinal cortex). The authors reported that while neurons in the deep layers of these structures exhibited an increase in activity in concomitance with hippocampal sharp waves, no theta modulation of their firing was ever observed. Only superficial neurons showed theta modulated firing, despite the fact that it is possible to record a clear theta field oscillation in both the superficial and deep layers of all these retrohippocampal structures.

The fact that TD cells, whose firing is so powerfully theta modulated, could be recorded from the deep layers of presubiculum, challenges the view that such a clear-cut distinction exists in the electrophysiological properties of deep vs superficial neurons in the presubiculum. Moreover, some theta modulated cells were recorded from the deep layers of the entorhinal cortex (Frank et al, 2000, 2001), further supporting that no clear demarcation between deep and superficial layers of retrohippocampal structures can be drawn on the basis of theta modulated firing.

The discharge probability of TD cells is maximal just before the trough of the locally recorded theta oscillation. TD theta preference is very similar to that reported for hippocampal pyramidal interneurons by Csicsvari et al (1999). In this study the authors recorded from hippocampal pyramidal cells and interneurons. Interneurons were separated in two categories, depending on their anatomical location, into: pyramidal interneurons, and alveus and stratum oriens interneurons. Theta oscillation was recorded locally (there is no difference in the phase of theta waves recorded from the alveus, stratum oriens and stratum pyramidale). The highest discharge probability of pyramidal interneurons was found to occur just before the trough of the locally recorded theta, that of alveus stratum oriens interneurons coincided with the trough, while that of pyramidal neurons lagged behind that of the interneurons, occurring in the ascending phase of the theta oscillation.

The fact that TD cells have a strikingly similar phase preference (to the locally recorded theta oscillation) to that of hippocampal interneurons, suggests that throughout the hippocampal-parahippocampal network similar physiological mechanisms underlie the emergence of global oscillatory patterns.

Unlike PC cells, TD cells show a very small variability in their phase preference distributions (even within cell, see fig. XII.3). As commented before, this makes it unlikely that firing of TD cells is subjected to phase shift, like that of PC cells. Because TD cells were never recorded on the linear track, where it is easier to detect phase shift, no definite answer to the question of whether TD cells show phase shift can be provided.

Chapter XIII

Topodirectional cell firing in differently shaped environments

We recorded the activity of topodirectional cells in differently shaped environments, in order to understand how environmental features determine topodirectional locational firing and to compare the results obtained with those of place cells. The directional activity of topodirectional cells was unaffected by these geometric manipulations. We also assessed the stability of topodirectional locational firing across similarly shaped environments, complementing the analysis of the stability of their directional properties presented in chapter XI.

Methods

The environments used were: 1) a cylindrical environment (cylinder), 2) a square environment (square) and 3) an unbounded circular platform (open platform). All three environments were placed within the curtained enclosure of the laboratory. Great care was taken to ensure that the environments were all centred within the curtained enclosure. The average distance between the centres of the environments for the trials included in the analysis was 2.50cm (±0.20), just over 1 bin (2.32 cm). This suggests that it is possible to compare directly the intertrial locational firing patterns, without needing to realign the maps.

38 TD cells were included in this study, recorded from 7 rats (rat a = 21, b = 5, c = 1, d = 2, e = 2, f = 6, g = 1).

All three conditions were tested for 10 cells, the cylinder and square conditions were tested for 7 cells, the cylinder and open platform for 14 cells, and the remaining 7 cells were recorded in the cylinder only. The cylinder condition was always tested at least twice (circle 1 and circle 2), to estimate the intrashape variability in topodirectional locational firing. Sometimes cells were recorded in the same shape during multiple trials, but only one of these trials for the square and open platform and two for the cylinder conditions were included in the analysis presented. Trials were selected as follows: the cylinder trial where the cell fired the highest number of spikes was chosen (baseline cylinder), along with the temporally closest trials in the square, open platform and the second cylinder. The firing rate maps for the square and open platform trials were topologically transformed into cylinder maps (see general methods section for details). Locational firing patterns in the baseline cylinder were then compared to all the other conditions (transformed square, transformed open platform and second cylinder), using a bin-by-bin correlation procedure which is described in detail in the general methods section of the thesis (chapter VI, p.72). The ANOVAs were performed on the r-values both before and after a Fisher Z transform. Only the results of the ANOVAs on untransformed r-values are presented, as no difference was observed between the transformed and untransformed conditions.

A separate analysis was also performed including all the trials ever recorded for each cell, by averaging the correlation values within each comparison (baseline cylinder- 2nd cylinder, baseline cylinder - transformed square, baseline cylinder - transformed open platform). The results are not presented here because they do not differ in any respect to those derived by selecting one trial per condition.

Locational firing maps were also compared by considering the distance in cm between the peak firing bins in the different shapes. This was done to complement the correlation study, given that the correlation method has some disadvantages (for example low correlation values are usually obtained for small locational fields, as compared to bigger fields, given the same shift in position).

Results

Intrashape stability of topodirectional locational firing

Topodirectional locational firing is stable when environmental geometry is not altered. Looking at the locational maps presented in fig XIII.2 it is clear that the locational fields do not shift or change shape in conditions baseline cylinder and 2nd cylinder. The correlation indices between firing rate maps of two cylinder (for 34 cells), two square (6 cells) and two open platform (11 cells) trials were computed. The boxplots and bar graphs of the distribution of correlation values obtained are presented in fig XIII.1. The average correlation values were 0.54 (± 0.04) for the cylinder, 0.53 (± 0.13) for the square and 0.47 (± 0.06) for the open platform. These values did not differ significantly [$F(2,48) = 0.359$, $p = 0.7$].
This result provides the grounds for the subsequent inter-shape analysis and confirms that topodirectional firing is stable across perceptually similar environments. This nicely matches the analysis of the directional firing of TD cells presented in chapter XI.

Topodirectional firing across differently shaped environments

Directional component

The preferred direction and directional selectivity were calculated for each TD cell and compared between 1) baseline cylinder and square, and 2) baseline cylinder and open platform trials.
In all cases there was no significant difference between the values, as assessed by pairwise two-tailed t-tests.

The mean difference in directional selectivity between cylinder and square was –0.78 ± 0.47 ($df = 16$, $t = -1.67$, $p > 0.1$), while the mean difference in preferred direction was –5.88° ± 13.29 ($df = 16$, $t = -0.44$, $p > 0.1$).

The mean difference in directional selectivity between cylinder and open platform was 0.41 ± 0.24 (df = 22, t = 1.70, p > 0.1), the mean preferred direction difference was 0.26° ± 9.98 (df = 22, t = 0.03, p > 0.1).
These results show that the directional tuning of TD cells is not affected by the geometry of the environment (across the changes considered), and that any change in locational field described below cannot be attributed to an alteration of the cell's directional properties.

Locational component

Fig XIII.2 shows the firing rate maps for 8 cells across the three environments tested. Both the raw and topologically transformed maps for square and open platform trials are presented to show how the topological transformation shapes the maps into cylinders.

The general pattern that emerges form this picture is that while locational fields are broadly similar in the cylinder and the square, the open platform firing patterns are generally different from those of the cylinder.

There are exceptions to this general trend, for instance, the firing pattern of cell TD f3 is different in each shape considered: its locational field shifts abruptly and duplicates in the square, one of these fields is present in the open platform. In the case of cells TD e3 and f1 firing patterns across the three shapes are very similar.
Figures XIII.3-4 show more examples of cells recorded in the cylinder and either the square or the open platform. Again, in most of the cases the cylinder and square firing are very similar (with the possible exception of cell TD g1 and TD b3), while those of cylinder and open platform are almost always different (but see TD a27 and c1).
The results of the correlation analysis confirm these intuitive considerations.

The average correlation values between cylinder and square trials was 0.33 ± 0.03 (n = 17), that between cylinder and open platform was 0.16 ± 0.04 (n = 24). The correlation values relative to cylinder-square comparisons are thus intermediate between those of cylinder- cylinder and cylinder-open platform (see fig XIII.5A). An ANOVA showed that the main effect of condition on the correlation values is significant [$F(2,72) = 28.72$, $p < 0.001$]. *Post-hoc* Scheffe's tests revealed that each condition differed significantly from each other ($p < 0.05$).

In summary, the results of the correlation analysis indicate that the degree of similarity between topodirectional cell firing patterns is: high in similarly shaped environments, lower across geometrically different bounded environments (cylinder and square), and almost zero across the circular walled and unbounded environments (cylinder and open platform). This suggests that the walls of the environment are strong determinants of topodirectional locational firing.

Fig XIII.5B shows the correlation values for each condition, providing a cell by cell breakdown. Cells are arranged in the temporal order they were recorded from, and the animal from which they were recorded is indicated at the bottom of the graph. No appreciable difference is visible in correlation values across animal or across

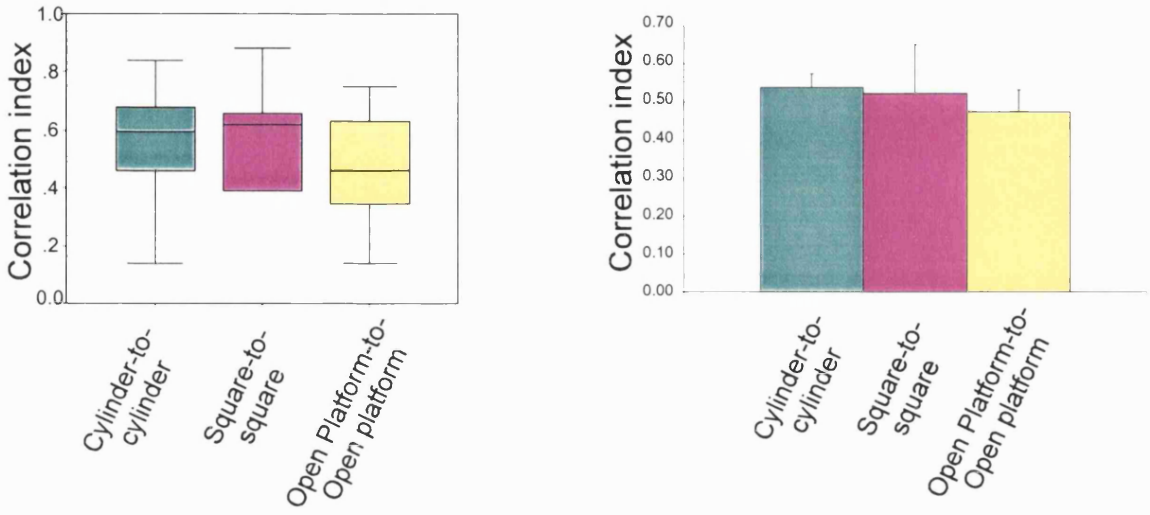
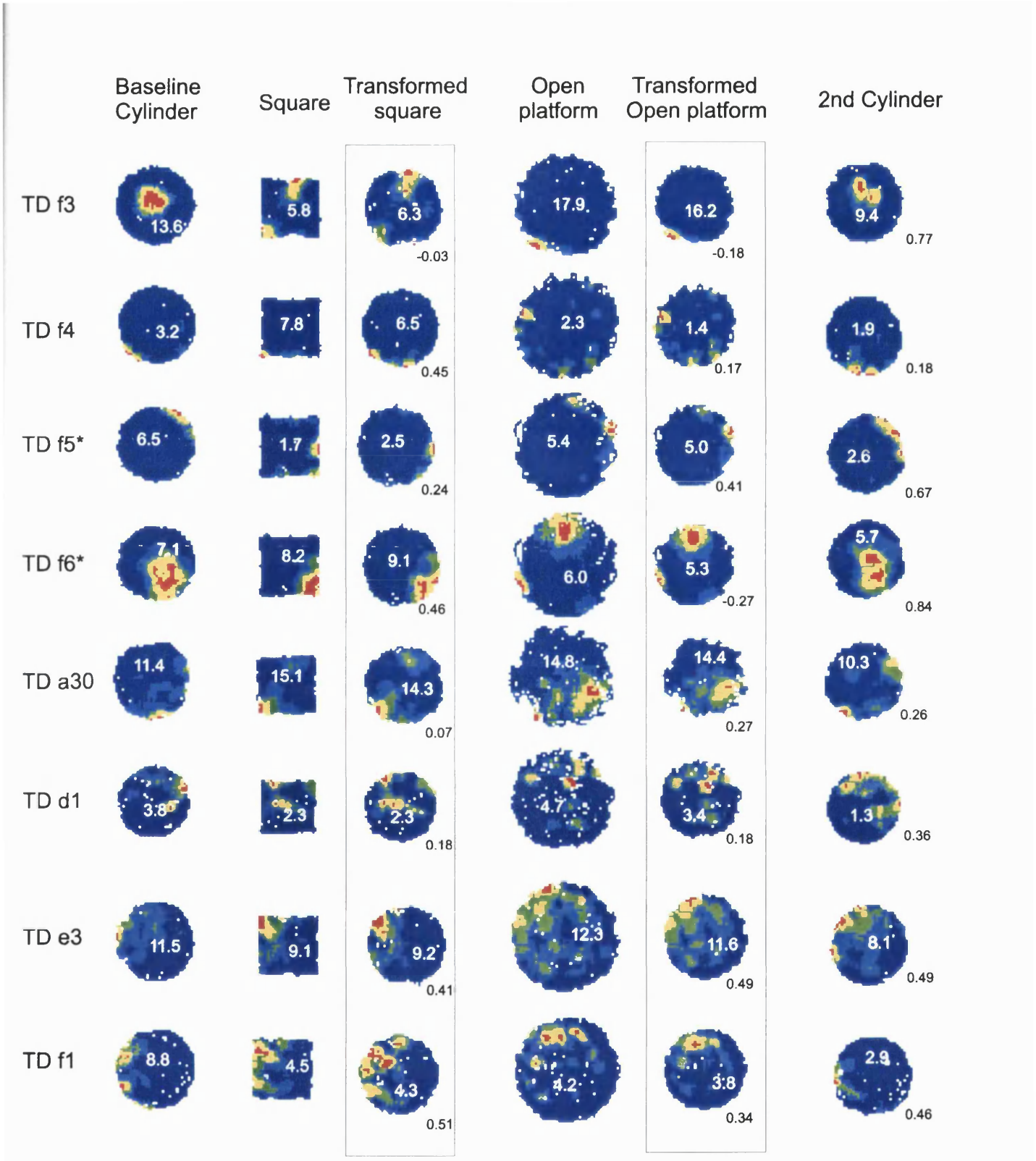


Fig XIII.1 Box plot (left) and mean and standard error plot (right) for each of the three identity-shape correlations.

These plots show that the averages of, and variability within, the correlation values for each type of identity shape correlation are broadly similar; there are no significant differences between the types. The mean and median of the Open-platform-to-open-platform correlations are somewhat lower than those of the other two, though far from being statistically significantly so. This is perhaps expected - the open platform is more of an uncontrolled environment in terms of the repeatability of behavioural and motor patterns, such as leaning over, and running at, the edges of the platform.

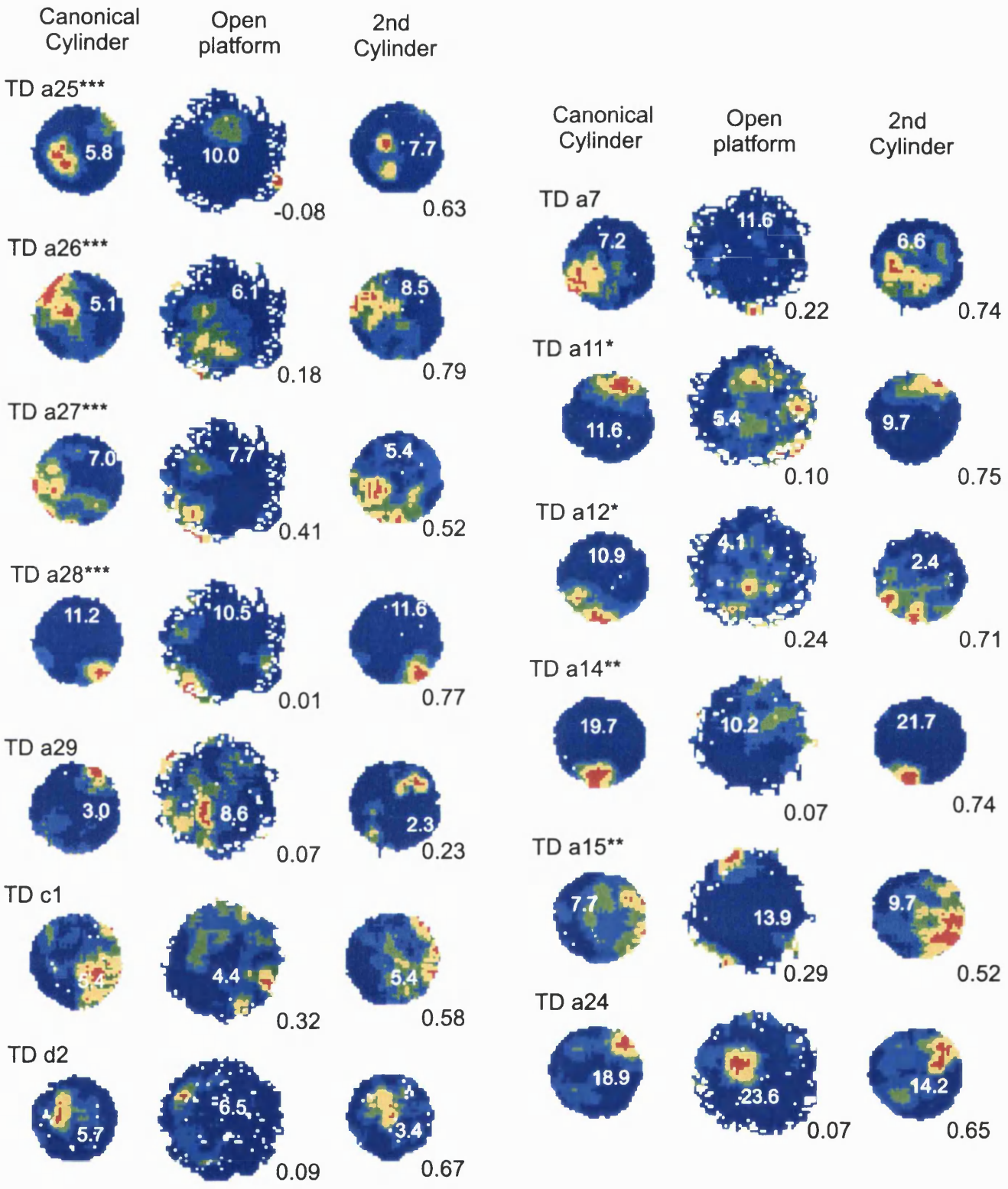
These data constitute useful baselines by which to test for across-shape correlations. For simplicity, this thesis presents the data comparing across-shape differences against the most consistent same-shape baseline, the cylinder-cylinder correlations.



XIII.2 Firing rate maps for eight topodirectional cells comparing locational firing in each of the three different environments (Cylinder, Square, and Open platform).

For each cell, four untransformed, and two topologically transformed, firing rate maps are presented, ordered according to shape, not recording sequence in the day. Canonical cylinder trial is that which contains the highest number of spikes. Square, Open platform and 2nd cylinder are the temporally closest trials, in these environments, to the canonical cylinder trial. For Square and Open Platform, topologically transformed maps are shown to right of untransformed maps in the given environment.

Correlation values compared to canonical cylinder are shown bottom right of transformed square, transformed open platform, and 2nd cylinder, firing rate maps.



XIII.3 Firing rate maps for thirteen topodirectional cells comparing locational firing across the Cylinder and Open circular platform.

For each cell, three untransformed firing rate maps are presented, ordered according to shape, not recording sequence in the day. Canonical cylinder trial is that which contains the highest number of spikes. 2nd cylinder and Open platform are the temporally closest trials, in these environments, to the canonical cylinder trial.

Correlation values compared to canonical cylinder are shown bottom right of the Open platform, and 2nd cylinder, firing rate maps. Cells sharing similar number of asterisks were recorded simultaneously.

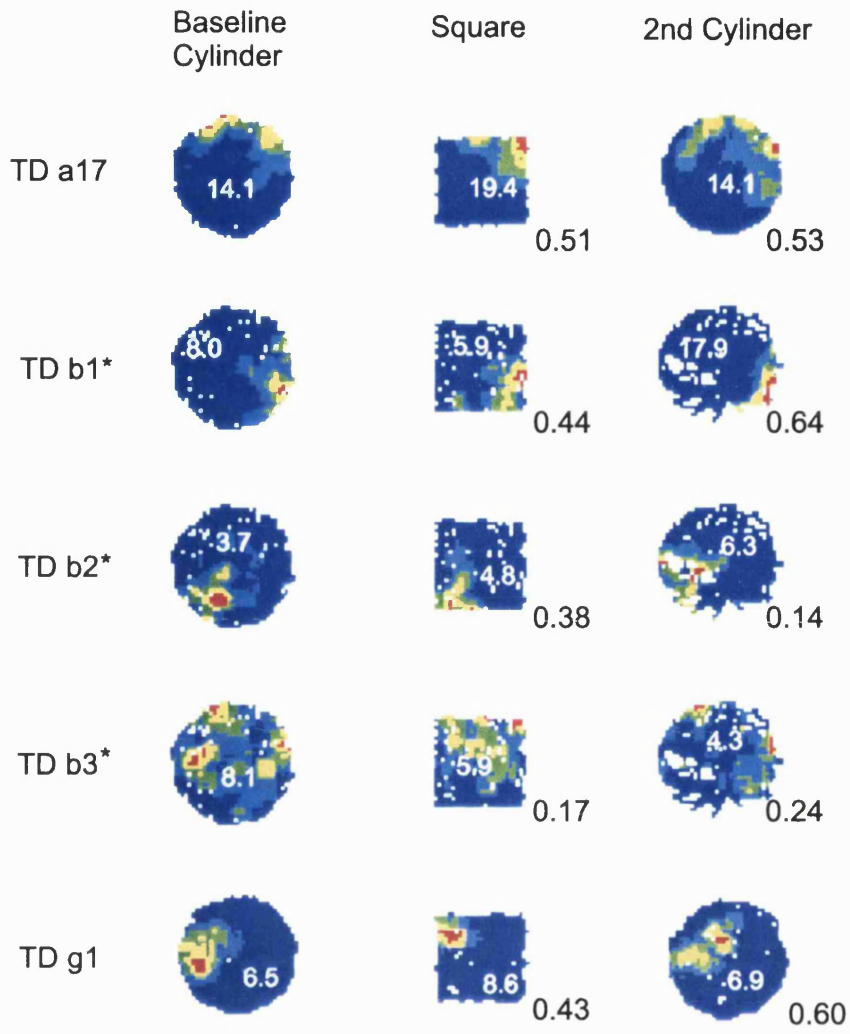


Fig XIII.4 Firing rate maps for five topodirectional cells comparing locational firing across the Cylinder and Square.

For each cell, three untransformed firing rate maps are presented, ordered according to shape, not recording sequence in the day. Canonical cylinder trial is that which contains the highest number of spikes. 2nd cylinder and Square are the temporally closest trials, in these environments, to the canonical cylinder trial.

Correlation values compared to canonical cylinder are shown bottom right of the Square, and 2nd cylinder, firing rate maps. The three asterisked cells were recorded simultaneously.

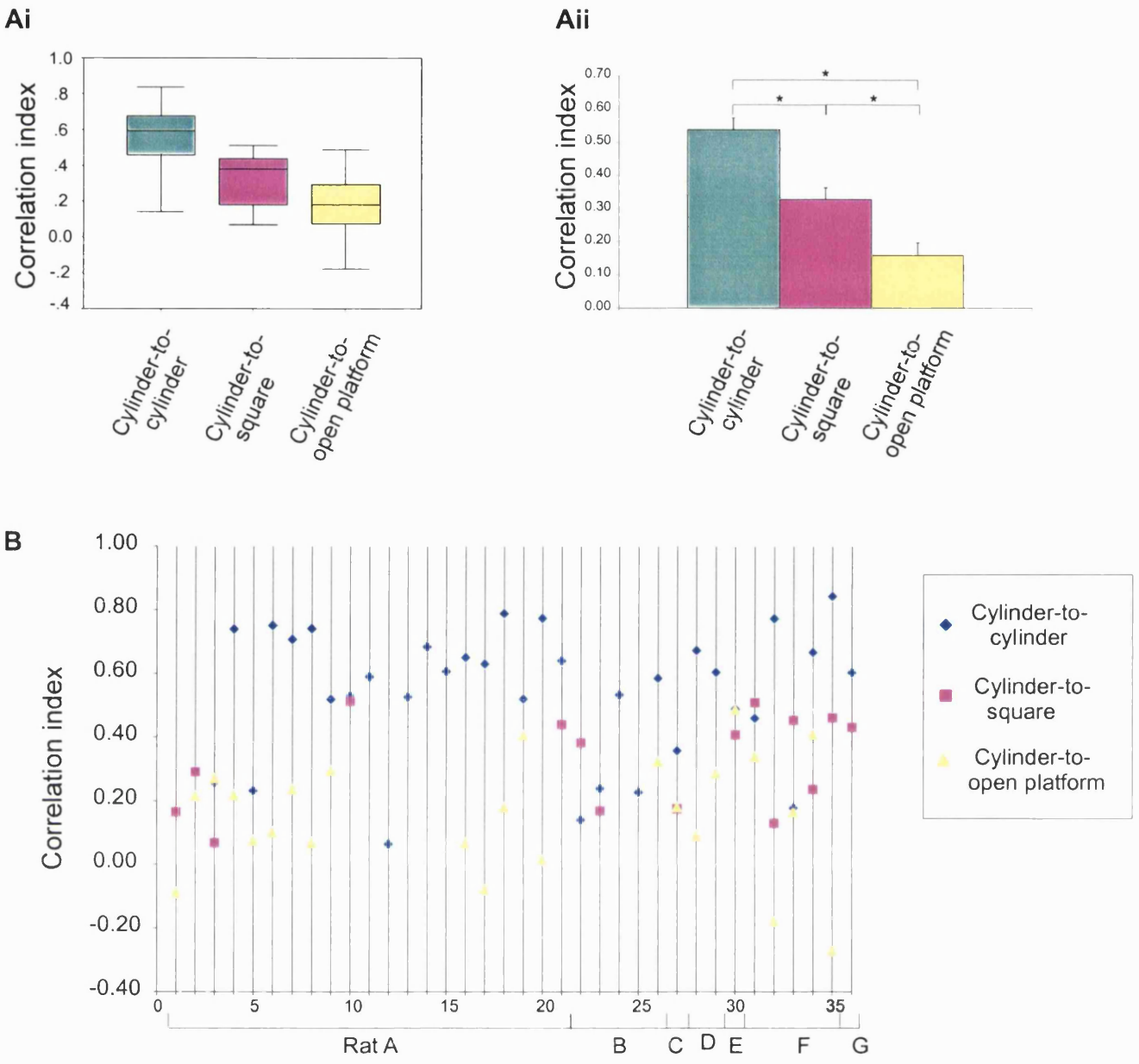


Fig XIII.5 Ai) Box plot and Aii) mean and standard error plot for each of the three types of environmental correlation. These are: 1) Cylinder to cylinder, 2) Cylinder to square, and 3) Cylinder to open platform.

It can be seen that the three types of environmental comparison show different ranges of correlation values, though roughly equal variability within these. The Cylinder to cylinder comparison used as the baseline shows the highest correlations, the Cylinder to open platform comparison show the lowest correlations, while the Cylinder to square correlations are intermediate between these two.

This same pattern of similarity and difference between the environments is also seen in the Distance-between-Peaks measure of similar locational firing.

As elsewhere, * reflects statistically significant differences at $p < 0.05$.

B) Graph showing the correlation values in the three environmental conditions across cells/animals. Each vertical line represents a cell. Horizontal lines at the bottom indicate the animal the cells were recorded from. Cells are arranged in temporal order of recording (left-earlier, right-later). The correlation values for the cylinder to cylinder comparison are represented as blue rombi, cylinder to square as purple squares, cylinder to open platform as yellow triangles.
No clear pattern is evident in the correlation values across either cells or animals.

recording time. It is interesting that recording time seems to have no effect on the correlation values across shapes. This could be due to the limited cell sample or it could reflect the fact that time/experience of the environments does not alter topodirectional environmental representations, in contrast to what is seen with hippocampal place cells.

In addition to the correlation study presented, a separate analysis designed to assess the similarity of TD firing across shapes was performed. This was done by calculating the distance in centimetres between the peaks of the locational fields in the different environments. The pattern of results is similar to that of the bin-by-bin correlation method. The mean distance between peaks across the two cylinder conditions was 21.1cm ± 2.9 (n = 34), between cylinder and square 27.0cm ± 3.9 (n = 17), between cylinder and open platform 32.8cm ± 3.2 (n = 24). An ANOVA revealed that there is a significant effect of condition on the distance between peaks [$F(2,72) = 3.653$, $p < 0.05$]. Note that there is an appreciable degree of variability in these measures, due to the fact that TD locational fields are more distributed and less compact than those of place cells. Despite this variability there is a clear pattern whereby the distance between the field peaks increases across the three conditions considered, confirming and strengthening the results obtained in the correlation study.

Discussion

The results can be summarised as follows: 1) directional and locational correlates of TD firing are stable across recording sessions, when environmental geometry is not altered; 2) the preferred direction of TD cells is not altered by changes in the geometry of the recording environment; 3) the same geometric alterations induce changes in the locational correlates of TD cells. In particular, the changes in the locational fields are more profound when the walls of the environment are removed (in the open platform condition) as opposed to just replacing the canonical cylindrical environment for a square one.
Thus, geometric changes of the recording environment selectively affect the locational correlates of TD cells, leaving their directional correlates unchanged.

It has been reported that HD preferred directions change between a cylindrical and a square environment (for 4/8 cells tested in Taube et al,1990b). This result was probably due to the fact that the animals were always disoriented before being placed in the recording environments.

In our experiments, rats were never intentionally disoriented, and extra effort was put into ensuring that no unintentional disorientation occurred (for instance, rats were always brought into the curtained enclosure in a standard way). HD cells were recorded under these conditions and no changes in their preferred directions were ever observed between the cylinder, square and open platform environments (data not shown). We speculate that the directional input to TD cells derives from the HD system, either directly from the presubiculum, or from those brain structures which are part of the HD system, and are anatomically connected to the pre- and parasubiculum. If the directional input of TD cells were presubicular HD cells, it would be unsurprising that TD preferred directions do not change in the cylinder, square and open platform.

The geometry of the recording environment has a stronger influence on the locational fields of TD cells. Both the position and the shape of TD locational fields change when environmental geometry is altered. This shows that TD directional and locational responses are dissociable. A dissociation between the locational and directional fields of TD cells was also seen when cells were recorded on the holding platform outside the curtained enclosure and in the cylinder inside it. In general, locational fields of TD cells changed abruptly between the holding platform and the cylinder (data not shown).

TD locational firing patterns across the cylinder and square are generally similar, and definitely more so than those across cylinder and open platform. This indicates that walls are an important determinant of TD locational firing. The importance of walls on TD firing has also been confirmed by a series of experiments, presented in the next chapter, where barriers were introduced in the recording environment (see chapter XIV, p. 122-124).

Place cells recorded in this laboratory, under very similar experimental conditions, in cylinder, square and open platform, show a similar pattern of results to those observed in TD cells. When rats have had limited exposure to the environments, place fields are similar across cylinder and square and totally "remap" (change position and shape) between the cylinder and open platform. Over subsequent exposures, hippocampal place representation of cylinder and square diverge (Lever et al, 2002, see chapter IV, p. 32).

Unfortunately, the amount of experience the animals received in the square and cylindrical environments was not carefully controlled in the TD experiment. The amount of experience in the square environment was in all cases rather limited, and certainly not comparable to that received by animals in the Lever et al (2002) experiment. For example, animal F (from which several cells were recorded in both the cylinder and square) received 18 trials in the cylinder and 6 in the square, over a period of 7 days. Over a similar amount of time, the animals in the Lever et al (2002) study received 21 trials in each shape. Even allowing for the strong inter-animal variability observed by Lever et al (2002), it is possible that the lack of divergence in the TD representations of square and cylinder (see fig XIII.3,4 and 5B) could be due to lack of sufficient experience in the two shapes. It is possible that, given more experience, TD representations of square and cylinder would have diverged, as was observed in PC cells.

Like TD cells, subicular and medial entorhinal neurons showed similar locational firing patterns in square and cylinder (Sharp et al, 1997; Quirk et al, 1992, see chapter IV, p. 36-37). In all the experiments conducted on these cells though, the amount of experience the animals received in each shape was not controlled, and hippocampal place cells were generally recorded from different animals from those where medial entorhinal and subicular cells were obtained. It is therefore possible that, exactly as is suggested for TD cells, given a sufficient amount of experience, medial entorhinal and subicular representation of square and cylinder would diverge.

Given the confound of experience, it is not possible to compare the results of geometric alterations on locational firing of medial entorhinal, subicular, topodirectional and hippocampal place cells.

More experiments, where the amount of exposure to the environments would be carefully controlled, are warranted to answer questions like: would the square and cylinder representations diverge in each of these structures over time/repeated exposures? Is the amount of experience required for this divergence to occur similar across areas?

These questions could help to identify functional relationships among the wide hippocampal locational system, and disentangle the contributions of each of the brain areas considered (hippocampus proper, subiculum, entorhinal cortex, pre- and parasubiculum) to the representation of location in the rodent brain.

Chapter XIV

Other environmental manipulations

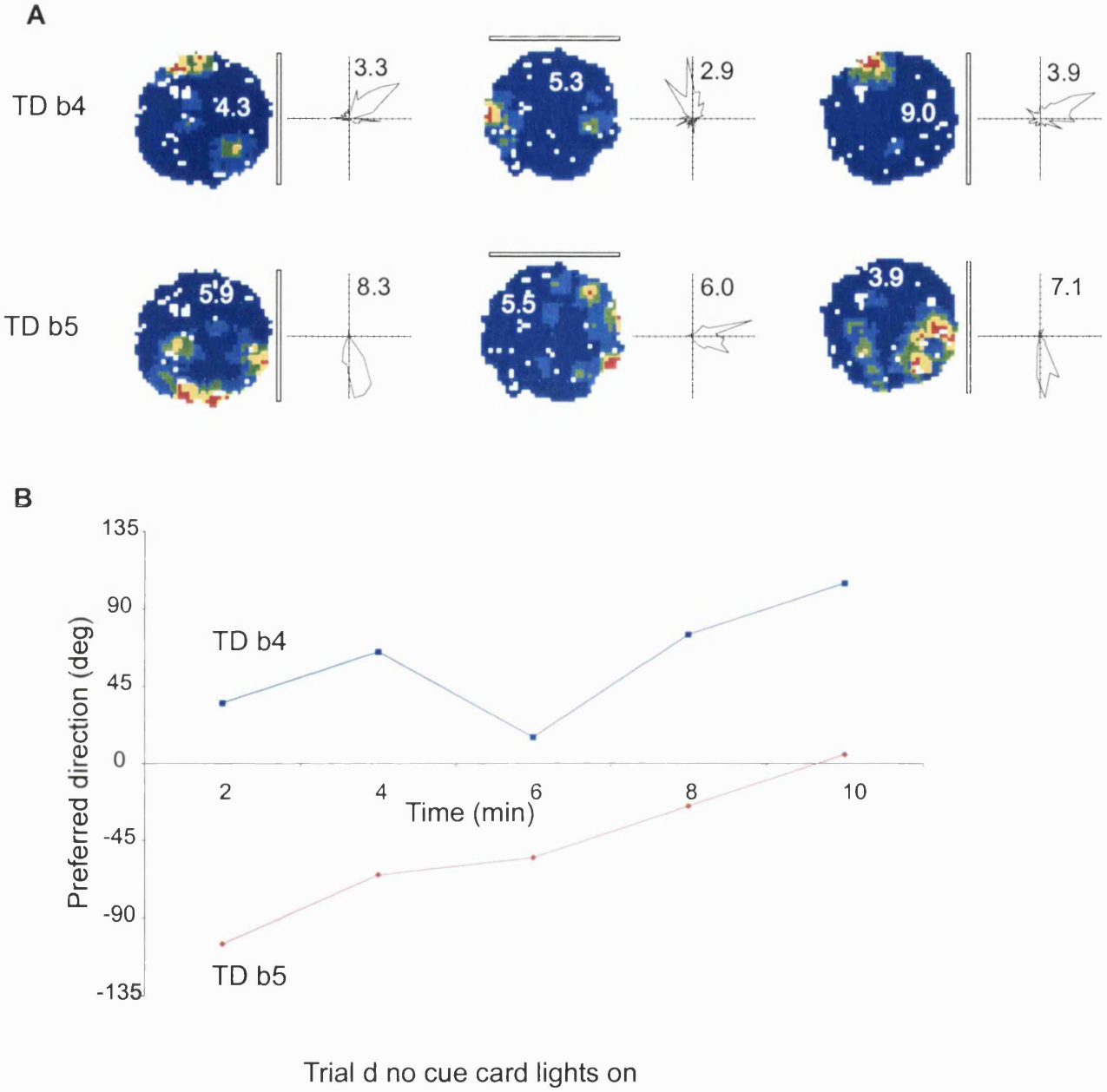
This chapter reports the effects that a variety of environmental manipulations have on topodirectional firing. In particular, three different types of experiments will be described: 1) cue card control experiments, where the position of the cue card is rotated within the curtained environment; 2) dark trials, where TD and HD cells are recorded in total darkness; 3) barrier experiments, where physical barriers are introduced in the recording environment. Small groups of cells are considered in all cases, allowing for only a descriptive analysis of the results.

Cue control

In the introduction to this thesis we described how the firing of both place and head direction cells can be controlled by visual landmarks. Classically, to observe this phenomenon the cue card is rotated within the curtained environment and cell activity is monitored in the standard and rotated conditions. Both place fields of place cells and preferred directions of head direction cells rotate by an amount nearly equal to the cue card. The animal is generally disoriented (by slowly rotating it in an opaque container) before being brought into the cue controlled arena, so as not to introduce a conflict between idiothetic and visual information.

In the experiment described here, two TD cells were simultaneously recorded (TD b4-5). Two baseline conditions were run with the cue card in the standard configuration (conventional east, see fig XIV.1), one before and one after the cue rotation session, where the card was rotated 90°counterclockwise (from east to north). At no point during the experiment was the animal disoriented (given that in previous experiments it had been shown that cue control could be obtained, in relatively inexperienced animals, without resorting to disorientation, data not shown). During standard sessions the animal was introduced into the curtained enclosure from the south and placed in the middle of the cylindrical environment facing north. During the cue rotation trial the animal was introduced into the curtained enclosure from the east and placed in the middle of the cylindrical environment facing west. Thus, in the cue rotation session, both the position of the cue card and the animal's point of entry were rotated by an equal amount.

Locational and directional firing rate maps for all three recording trials are shown in fig XIV.1. Locational and directional fields of the two cells recorded rotated with the cue card rotation. The difference between the preferred direction in the cue rotation vs standard cue conditions was 70° for TD b4 and 100° for TD b5, while the difference in mean angle values were 76° and 74° respectively. This demonstrates that TD cell firing can be controlled by visual landmarks, very similarly to place and head direction cells. Moreover, the locational and directional components of TD firing rotate together, maintaining a constant relationship within each cell. Simultaneously



Trial d no cue card lights on

Fig XIV.1 Cue control over orientational firing of two simultaneously recorded topodirectional cells (TD b4 and TD b5).

A) Rotation by 90 degrees anticlockwise of the point of entry into, and the cue card within, the curtained environment, re-orients the preferred direction, and locational field of the two cells, by about this amount.

B) In the absence of the cue card, but under normal lighting conditions, the preferred direction of the cells drifts over time. The preferred directions of the two cells appear to shift together. (The outlying preferred direction at the four-six minutes period in cell TD b4 probably reflects the noisiness of the measure over two minutes, rather than genuine uncoupling of the two cells.)

recorded topodirectional cells rotate in unison, as is also found in place and head direction cells.

The fact that the cue card was the principal orientational landmark for rat b was confirmed by a further experiment. When the cue card was removed altogether from the environment the directional and locational fields of the cells rotated slowly counterclockwise. During the 8 min recording session they accumulated a total displacement of 100° (with a mean drift of 12.5° per minute). The way in which the mean angle of both TD cells changed during the course of the experiment is shown in fig XIV.1B. Again, both TD cells rotated in unison, maintaining a constant difference between their mean angles. Interestingly, in the absence of the cue card, though the lights were always on during the recording session, the TD system was unable to use the remaining visual information to maintain a stable representation of the environment. This is rarely seen in experienced animals. It could be explained by the fact that rat b had only been exposed three times to the curtained environment before the experiments just described, and therefore the TD system was not yet bound to a constellation of perceptual features, but only (or mainly) to the most prominent visual cue present in the environment.

Darkness

In order to assess the role that visual information plays in establishing and maintaining topodirectional signal, three TD cells were recorded, on separate occasions, in total darkness.

This section also includes the results of similar experiments conducted on HD cells, which support the view that visual information is necessary to maintain a stable head direction signal.

Topodirectional cell responses in the dark

The three TD cells recorded in the dark were TD g1, f3 and f6. In all cases cellular activity was recorded while the animal foraged in the cylindrical enclosure, within the curtained environment. All asymmetric cues (like the cue card) were removed from the curtained environment, and both the lights and the audio speakers were simultaneously turned off. No attempts were made at disorienting the animals, which were always brought inside the curtains in a standard way, similarly to what was described in the cue control section of this chapter. The results of the dark experiments are summarised in fig XIV.2-5.

In the case of cell TD g1 the animal was introduced in the recording environment when the lights were on, cell activity was recorded for a total of 21 minutes: during the first 12 minutes the lights were on, then off for 6 minutes (from 12 to 18), and then on again for the remaining 3 minutes (from 18 to 21). Fig XIV.2 shows the locational and directional maps and the paths taken by the animal during four recording epochs, each 3 minutes long. During the first and last epochs the lights were on, the two columns in the middle refer to data acquired in complete darkness. During the dark epochs there was a pronounced decrease in the cell firing rate (which

was reduced to roughly half of that during the light epochs). This decrease in firing rate cannot be easily explained on the basis of a change in the animal's behaviour, given that the paths taken by the rat during the light and dark condition do not differ in any systematic way.

The directional preference of the cell is not significantly affected by the absence of light, remaining almost completely stable during the 6 dark minutes (see fig XIV.2B which shows the values of the mean angle for the cell at 1 minute intervals during the experiment). Rat G had extensive experience of both the curtained enclosure and the cylindrical environment before the experiment described took place and this might explain why the directional preference of the cell remained stable in total darkness.

The locational field shifts towards the south west edge of the circle during the dark period, returning back to a more central-west position in the subsequent light epoch. This shift in position suggests that under the total absence of visual information, topodirectional signal is possibly dominated by tactile and/or vibrissal inputs, which mostly drive the cell near to the cylinder's wall.

Both cells TD f3 and f6 were recorded in the dark following the procedures described in the methods section of this thesis (chapter VI, p. 66). Briefly, both the lights and the audio speakers were turned off 2 minutes before the animal was introduced in the curtained environment. Cellular activity was recorded for 20 minutes in total darkness. At five minutes from the start of the trial the rat was gently picked up and held above the floor for ten seconds, without displacing him. At ten minutes this procedure was repeated, but this time both the cylinder and the floor were rotated by 180°, the rat was put back on the floor, and recording continued for the remaining 10 minutes. The cylinder and floor rotation was performed to test if the directional system used tactile and/or olfactory cues attached to the floor or the wall of the cylinder to maintain a stable orientation. At the end of the 20 minutes, recording was suspended for up to three minutes to allow for the transfer of the data acquired from the PC to a SUN workstation. During this time the rat remained in the cylinder, in complete darkness. The subsequent recording session lasted for 14 minutes, the initial 4 in darkness and the last 10 with the lights turned on.

TD f3 was simultaneously recorded with a head direction cell (HD f2). Fig XIV.3 shows the locational and directional plots for cell TD f3 over four trials: trial 1 is an 8 min long baseline (lights on), trial 2 and 3 are the dark trials whose procedures have just been described and trial 4 is the subsequent baseline (8 min long, lights on). The cell stopped firing during both dark trials 2 and 3, and resumed its firing only during the next baseline session (trial 4). Somewhat surprisingly the cell did not start firing during the light epochs of trial 3 (last 10 minutes of the trial), but only after the rat was reintroduced in the curtained environment with the lights on (in trial 4).

Polar plots for the simultaneously recorded HD cell are shown in the same figure (fig XIV.3A). The preferred direction of the cell is north-east during the two baseline trials, and it changes abruptly by about 180° at the beginning of trial 2. This is probably because the rat was introduced in the curtained enclosure in total darkness. It is important to note that although rat F had extensive experience of the curtained enclosure before this trial took place, this was the first time that the lights were

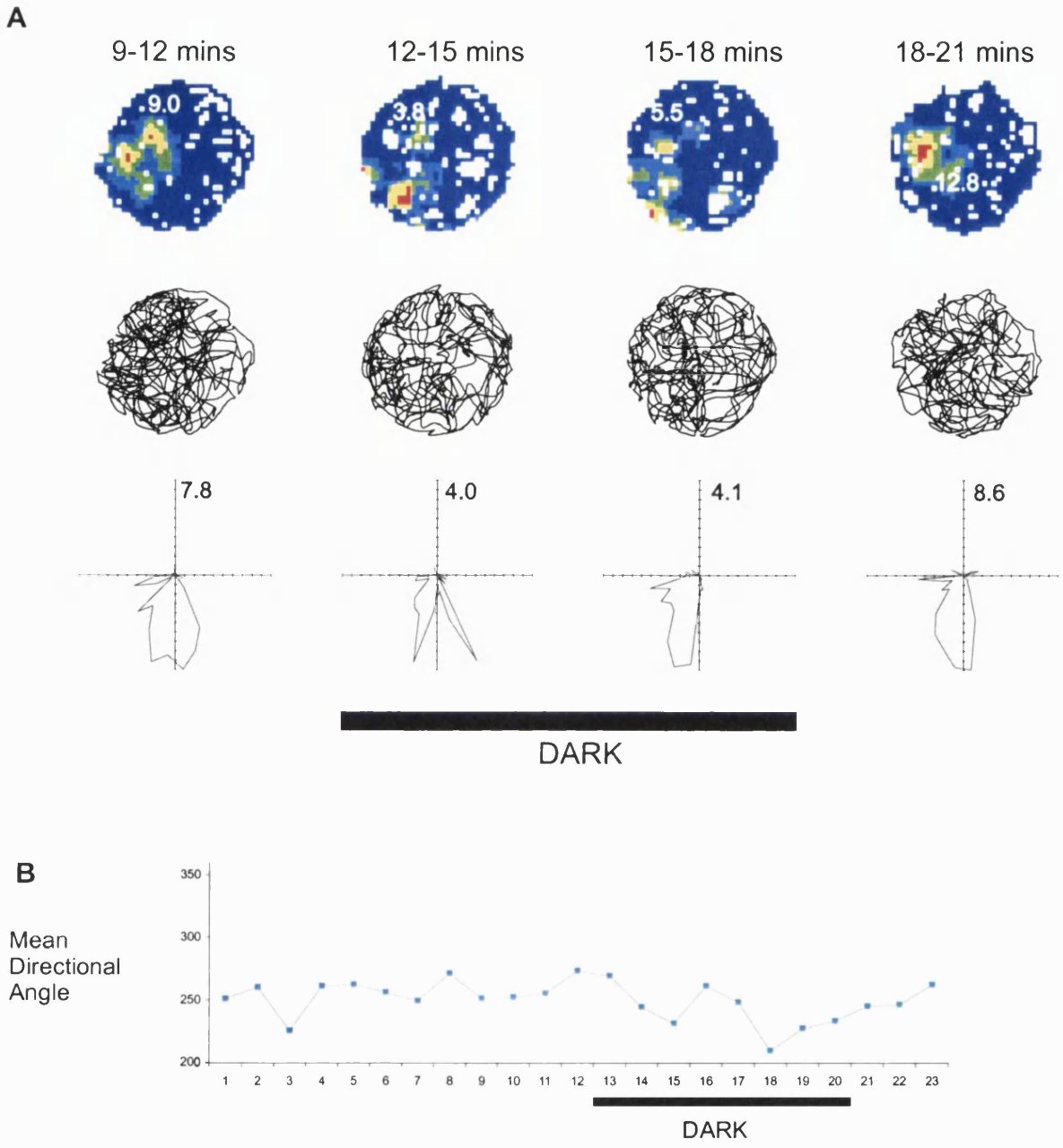


Fig XIV.2 The effect of darkness on a topodirectional cell (TD g1)

A) Locational and directional correlates of firing under normal light (1st and 4th) and dark (2nd and 3rd) conditions, presented in three-minute periods. Also shown under firing rate maps are plots of the paths taken by the animal.

Path length does not alter during dark conditions. The cell's firing rate drops during darkness, and the locational field moves closer to the edge of the cylinder. The orientational directional firing is basically unaltered.

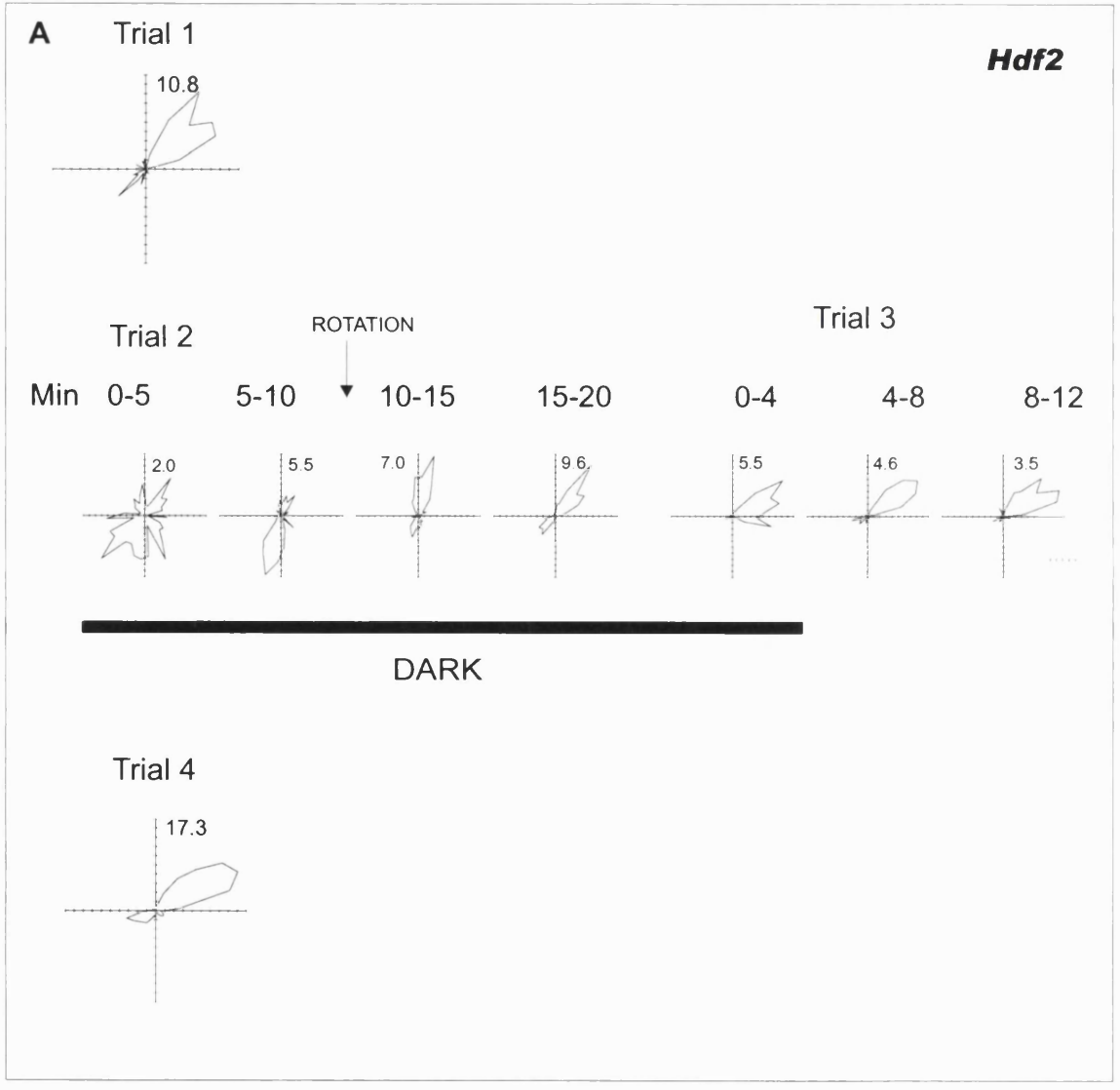
B) The mean directional angle, presented in one-minute periods. The orientational firing of the cell does not change by more than 60 degrees over the whole recording period. Darkness does not really affect cell's directional angle.

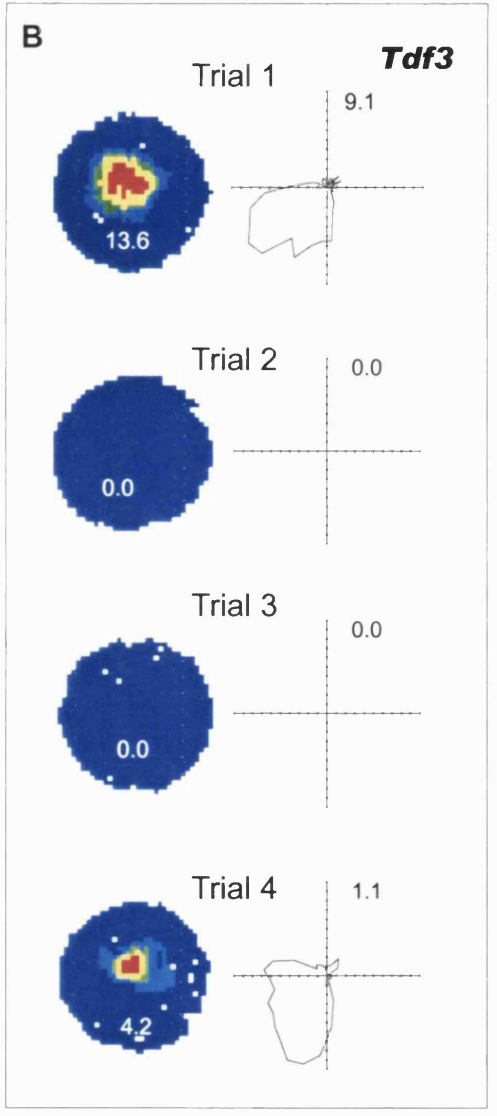
Fig XIV.3 Effect of darkness on simultaneously recorded topodirectional cell (TD f3) and head direction cell (HD f2)

Trials 1 and 4 are baseline sessions, lasting 8 minutes, during which the lights were constantly on. Trial2 was 20 minutes long, lights were off at all times. At 10 minutes during trial 2 the animal was lifted and the cylinder and floor rotated 180 degrees. The animal was left in the cylinder between trials 2 and 3. Trail 3 was 14 minutes long, during the first 4 minutes the lights were off, then were switched on for the remaining 10 minutes.

A) Directional plots of head direction firing during the 4 trials. Directional firing is stable across baseline sessions (trials 1 and 4). Upon entry in the cylinder, in darkness (trial 2), the preferred direction resets, rotating 180 degrees from the baseline value. The directional preference rotates with the floor+cylinder at ten minutes during trial 2, suggesting that, in the absence of visual input, the head direction system relies on olfactory/tactile cue present on the floor or walls of the cylinder. After the rotation the cell's preferred direction drifts slowly clockwise during the dark periods of both trial 2 and 3. When the lights are switched on at 4 minutes during trial 3, the preferred direction of the cell stabilizes.

B) Locational maps and directional plots of topodirectional cells during the four trials. Darkness (trial 3 and 4) totally suppress topodirectional firing. Somewhat surprisingly, topodirectional firing does not resume during the last 10 minutes of trial 3, when the lights are on. The cell starts firing again (though at a lower rate, only in the subsequent baseline trial (trial 4).


A
Trial 1
Hdf2
10.8
Trial 2
ROTATION
Trial 3
Min
0-5
5-10
10-15
15-20
0-4
4-8
8-12
2.0
5.5
7.0
9.6
5.5
4.6
3.5
DARK
Trial 4
17.3


B
Tdf3
Trial 1
9.1
13.6
Trial 2
0.0
0.0
Trial 3
0.0
0.0
Trial 4
1.1
4.2

switched off. The lack of experience of the dark condition might have prompted the reset of the directional system (and the total suppression of the topodirectional signal). The preferred direction of cell HD f2 is fairly stable during the first ten minutes of trial 2 and rotates with the cylinder and floor (180° at 10 min). In the remaining ten minutes the cell's preferred direction drifts only by 30°. In trial 3 the slow drift is still evident, until the lights are switched on at 4 min and the preferred direction of the cell "freezes" in the north-east direction. It is probably only by chance that the cell's preferred direction at the end of trial 3 is the same as that of the baseline conditions.

Fig XIV.4-5 show the results of a similar experiment. Again, one TD (f6) and one HD cell (f6) were recorded. This experiment took place 15 days after the one just described. During this period the rat was exposed to two dark recording sessions during which only HD cells could be recorded (in both cases the HD preferred directions drifted in darkness).

TD firing was not suppressed during the dark period, but a profound directional instability was observed for both the TD and HD cell. Interestingly, very similarly to what was found for cell g1, the place field of the TD cell shifted towards the edge of the circle when the lights were off. Arguably, during the last 4 minutes of trial 3 (lights on) the cell started firing in a more central position within the cylinder, in a pattern more resembling that of the baseline condition.

In conclusion, the results presented here suggest that visual information is crucial for the maintenance of topodirectional firing. In two of the three cases examined, either a strong decrease in firing rate, or a total suppression of firing were observed. In the two cells that continued firing during the dark periods (TD g1 and f6), the locational fields of the cells shifted towards the edge of the environment, indicating that tactile cues might be important in driving TD firing in the absence of visual inputs. This is very different to what has been reported for place cells, which are rarely affected by darkness (Quirk et al, 1990), and fits nicely with the anatomical findings which emphasize that presubiculum receives strong projections from a variety of visual areas (area 18b, restrosplenial cortex, see chapter I, p.10).
As to the directional instability of cell TD f6, it mirrored that of the simultaneously recorded HD cell, and it is very likely that it was caused by the instability in the HD system.

Head direction cell responses in the dark

HD cells from six animals were recorded in darkness. Cells recorded from 4 out of the 6 animals showed a pronounced directional instability in the dark (rats E, F, H, J), while cells recorded in the two remaining animals (rats K and L) were stable over 10 min dark recording sessions.

Fig XIV.6-7 show the results of eight separate experiments, illustrating the way in which the preferred directions of the HD cells recorded drifted over time (in 1 min intervals). In total 17 HD cells were recorded and in almost all the cases (with the exception of rat F day1 and rat J day3) more than one HD cell was simultaneously recorded.

The animals were never intentionally disoriented and they were introduced into the curtained enclosure in the standard way described in the previous section. The lights were already off for a couple of minutes before the animal was taken into the curtained enclosure and the recording environment was always the cylinder. No obvious asymmetric cues were present within the curtained enclosure.

The general pattern that emerges from fig XIV.6-7 is one of directional instability. In three cases (rat H 1st trial fig XIV.6A, rat j day3 fig XIV.6B, and rat E experiment 1 fig XIV.7A) the cells' preferred directions were stable to start with and started drifting only after some time (4 minutes for rat H, 10 minutes for both rat J and E). In the other cases presented the preferred directions start drifting almost immediately after the start of the trial. Usually the drift occurs only in one direction (increasing angles denote a counter clockwise rotation), but in two cases (rat F and rat J day 3) a clockwise rotation follows a counterclockwise one.

These results could be explained in terms of an accumulation of errors in the path integration system. In the absence of visual inputs, the directional system of the rats is heavily reliant on idiothetic information to update its orientation. The path integration mechanism is bound to accumulate error over time and this results in the preferred direction drift observed.

The speed of drift is different in each instance, but in all cases simultaneously recorded cells maintain the same angular difference between their preferred directions (rotating in unison).

We can now look in more detail at each single experiment. For rat H two temporally contiguous dark trials are shown (8 minutes each). During the intertrial interval the rat was brought outside the curtained enclosure, placed on the holding platform and the lights were switched on. Before this experiment took place, the rat had had extensive experience of both the curtained enclosure and the cylinder, and had been previously exposed to the dark condition. Four HD cells were simultaneously recorded. During the 1st trial (fig XIV.6A, trial 1), the preferred directions were stable for 5 minutes and then started rotating counterclockwise at an average speed of 43°/min (averaged across cells). In the subsequent trial (figXIV.6A, trial 2) the drift started earlier on, it was always counterclockwise, and its speed was 34°/min (over the whole trial).

In the case of rat F (fig XIV.6B), two HD cells were simultaneously recorded. Their preferred directions started rotating counterclockwise almost immediately, at an average speed of 20°/min, until 11 minutes through the trial, when the direction of rotation reversed, and the preferred directions started drifting clockwise, at the same speed (20°/min). This trial occurred 5 days after the one described in the previous section (when TD f3 and HD f2 were recorded, see fig XIV.3), and 10 days before the one where TD f6 and HD f6 were recorded (see fig XIV.4-5). This trial differed from those, because in this case the rat was simply introduced into the curtained environment in the dark, and recording lasted for 22 minutes; no cylinder + floor rotation was performed.

The case of rat J is more interesting (fig XIV.6C). The three experiments presented here represent the whole dark experience the rat had. Each experiment was

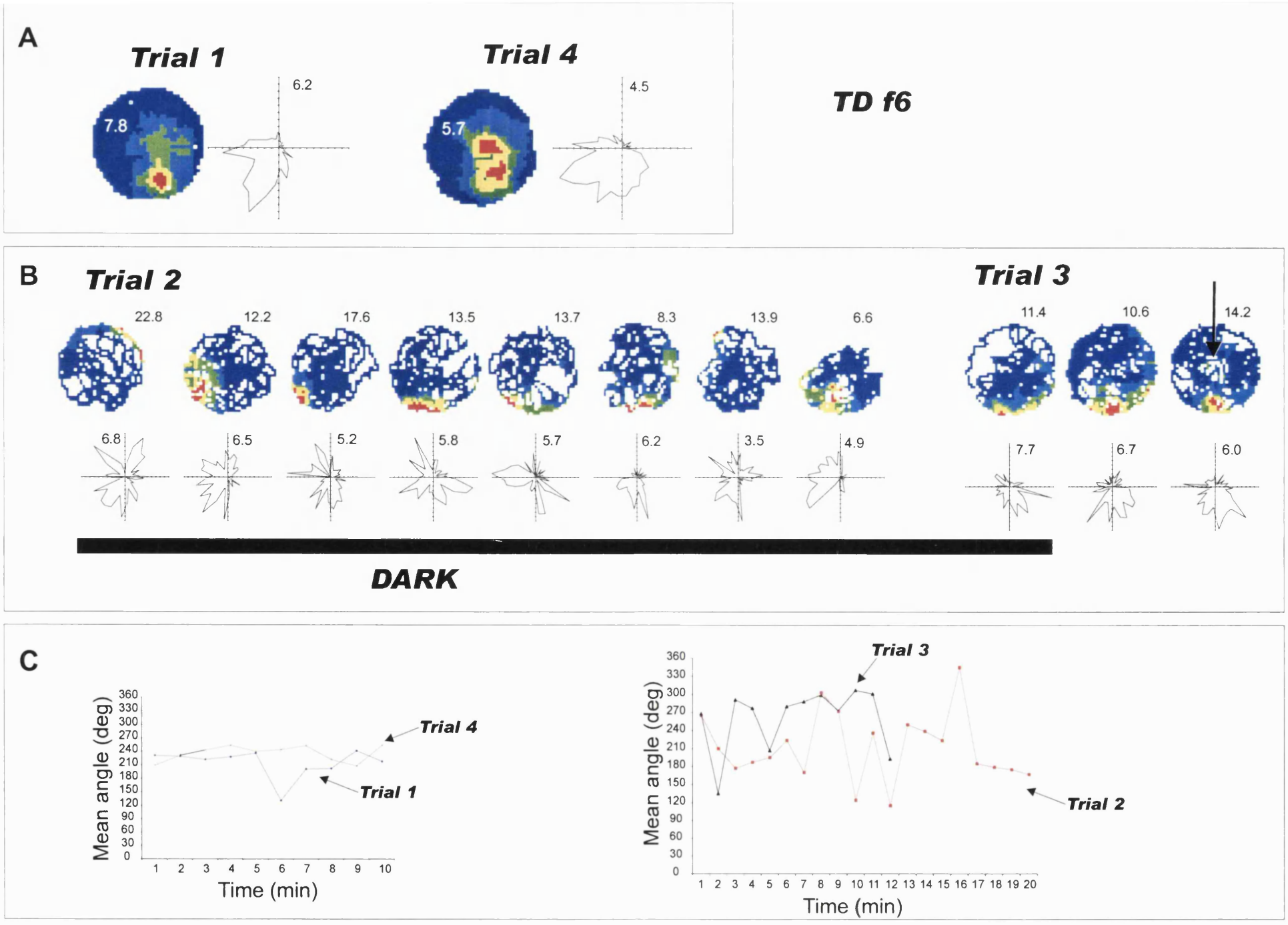
Fig XIV.4 Effect of darkness on simultaneously recorded topodirectional cell (TD f6) and head direction cell (HD f6, see fig XIV.5).

Trials are as in figure XIV.3

A) Firing rate maps and polar plots of topodirectional firing during the two baseline trials (trial 1 and 4). Firing is stable across baseline sessions.

B) Firing rate maps and polar plots of TD firing during trials 2 and 3. For trial 2 maps/plots refer to 2.5 minute long successive time intervals, for trial 3 to 4 minutes long intervals. Both the locational and directional correlates of cell TD f6 are highly unstable during the dark sessions. The locational field shifts towards the edge of the cylinder, suggesting that in the absence of visual inputs, information provided by the walls of the cylinder (possibly tactile) is needed to drive cell firing. Arguably, by the end of trial 3, after the lights have been switched on again, the cell starts firing in a more central position in the cylinder (see arrow).

C) The average angle is constant during both baseline trials (unfder lit conditions; left). A profound instability in directional firing is observed during both trials 2 and 3. During the last 10 minutes of trial 3 (when the lights are on) directional firing is more stable than during the preceding dark period (throughout trial 2 and from 0-4 minutes in trial 3).


A
Trial 1
Trial 4
7.8
6.2
5.7
4.5
TD f6
B
Trial 2
Trial 3
22.8
12.2
17.6
13.5
13.7
8.3
13.9
6.6
11.4
10.6
14.2
6.8
6.5
5.2
5.8
5.7
6.2
3.5
4.9
7.7
6.7
6.0
DARK
C
Mean angle (deg)
Time (min)
Trial 4
Trial 1
Trial 3
Trial 2

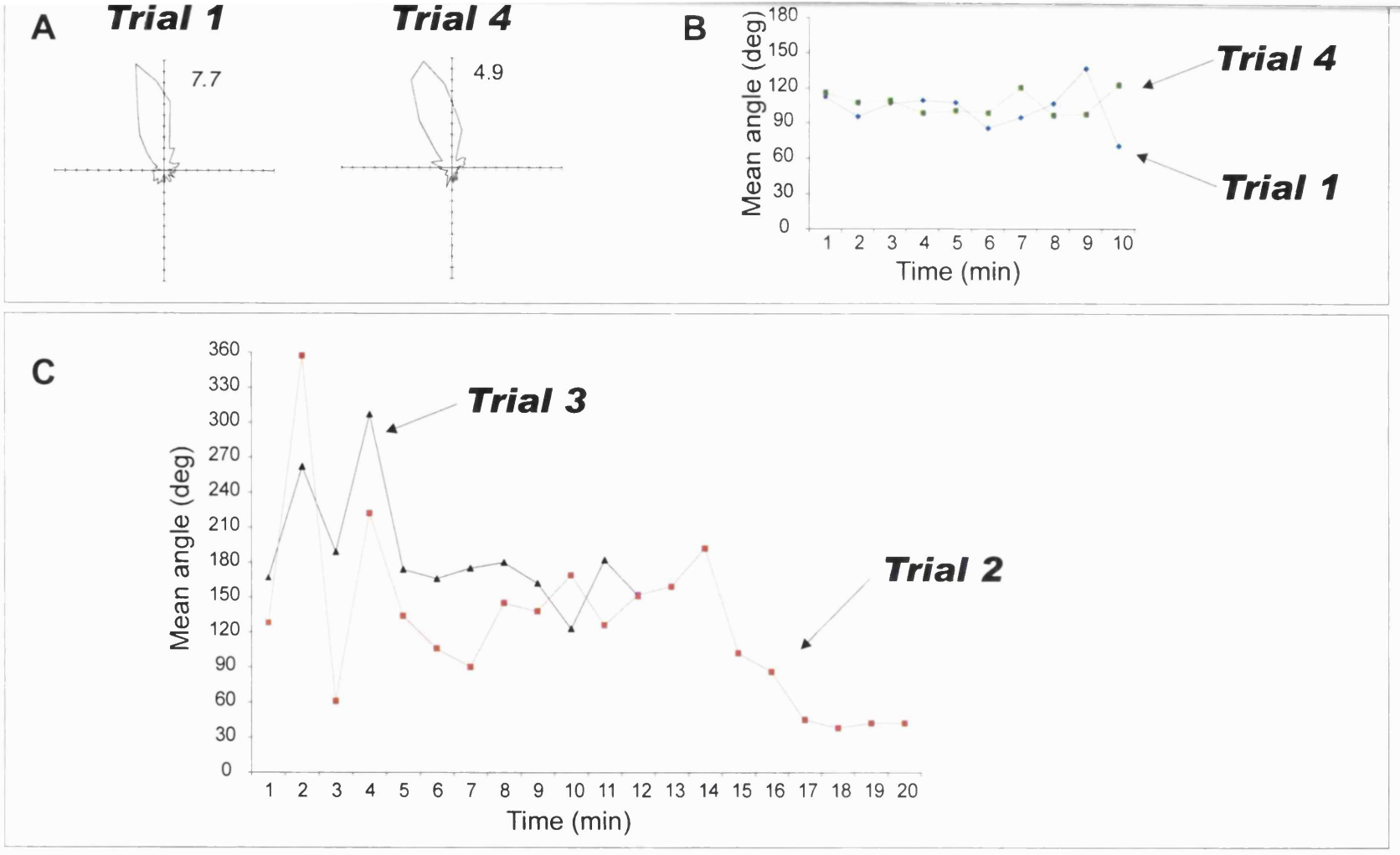


Fig XIV.5 Effect of darkness on simultaneously recorded topodirectional cell (TD f6; see fig XIV.4) and head direction cell (HD f6).

Trials are as in figure XIV.3

A-B) HD firing is stable across baseline sessions (Trials 1 and 4). Polar plots (A) and graph of mean angle vs time (B) for trails 1 and 4show that during baseline sessions (with the lights on) the directional firing of HD f6 is stable, similarly to what found for the simultaneously recorded TD cell (fig XIV.4)

C) Throughout trial 2 and during the first four minutes of trial 3, directional firing is not stable. Directional stability resumes upon switching the lights on, at 4 minute during trial 3.

Fig XIV.6 Head direction cells responses in the dark

All graphs show the values of preferred direction at one minute intervals. Black bars at the bottom of graphs indicate dark periods.

A) Four HD cells were simultaneously recorded in rat H (HD h2-5). Results from two successive 8min trials are shown. During the first trial directional firing was stable, and started drifting only after 5 minutes from the start of the trial (speed of drift 43 deg/min). During trial 2 the preferred directions started drifting slowly and then accelerated after 3 minutes from the start of the trial (average speed of drift 34 deg/min). All the HD cells simultaneously recorded rotated in unison.

B) Two HD cells were simultaneously recorded in rat F (HD f4-5). The preferred direction of both cells started drifting counterclockwise from the start of the trial (20deg/min) and at 11 minutes during the trial the direction of drift inverted (20 deg/min) and remained clockwise until the end of the trial.

C) Three experiments are shown for animal J. They represent the whole dark experience for the rat and they occurred at seven days intervals.

.

Experiments 1 and 2 consisted of two trials (the discontinuity in the preferred direction lines indicates the end of one trial and beginning of next trial). Between the two trials the animal was left in the cylinder in darkness. The first trial lasted 20 minutes and lights were always off. The second trial started approx. 3 minutes after the first and lasted 14 minutes: the first four minutes the lights were off, then on for the remaining 10 minutes.

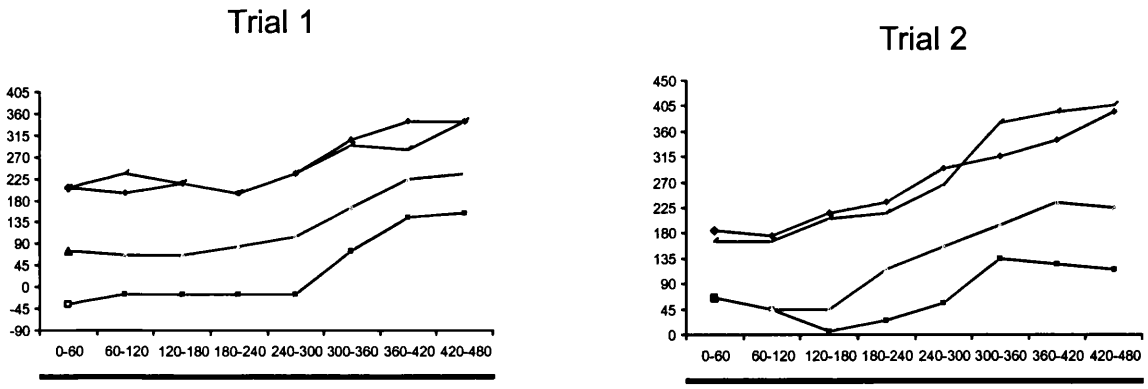
During experiment 1, three HD cells were simultaneously recorded (HD j9-11). Their preferred directions started drifting counterclockwise from the start of the first trial at an average speed of 46 deg/min. The drift slowed down by the end of trial 1 and during trial 2 the preferred directions were stable (before and after switching the lights on).

During experiment 2, 3 HD cells were simultaneously recorded (HD j14-16). The preferred directions started rotating counterclockwise from the beginning of the trial at an average speed of 43 deg/min. The drift slowed down during the last 6 minutes of the first trial, and continued at this slower pace during the first 4 minutes of the second trial, until the lights were switched on, and the preferred directions stabilised.

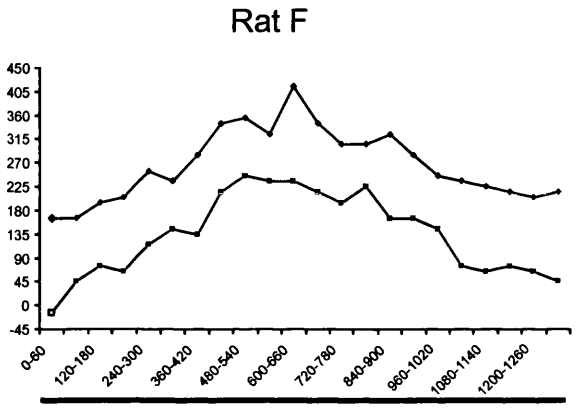
During experiment 3 only one HD cell was recorded (HD j17). The cell's preferred direction was roughly stable for the first 10 minutes of the first trial, then it started drifting counterclockwise at a speed of 27deg/min. Preferred direction stabilised when the lights were turned on and started drifting again when the lights were switched off (initially counterclockwise and then clockwise). The fact that the preferred direction was stable, in darkness, for about 10 minutes at the start of experiment 3 suggests that through subsequent dark experiences the HD system in rat J "learned" to cope, at least temporarily, with the absence of visual inputs.

A

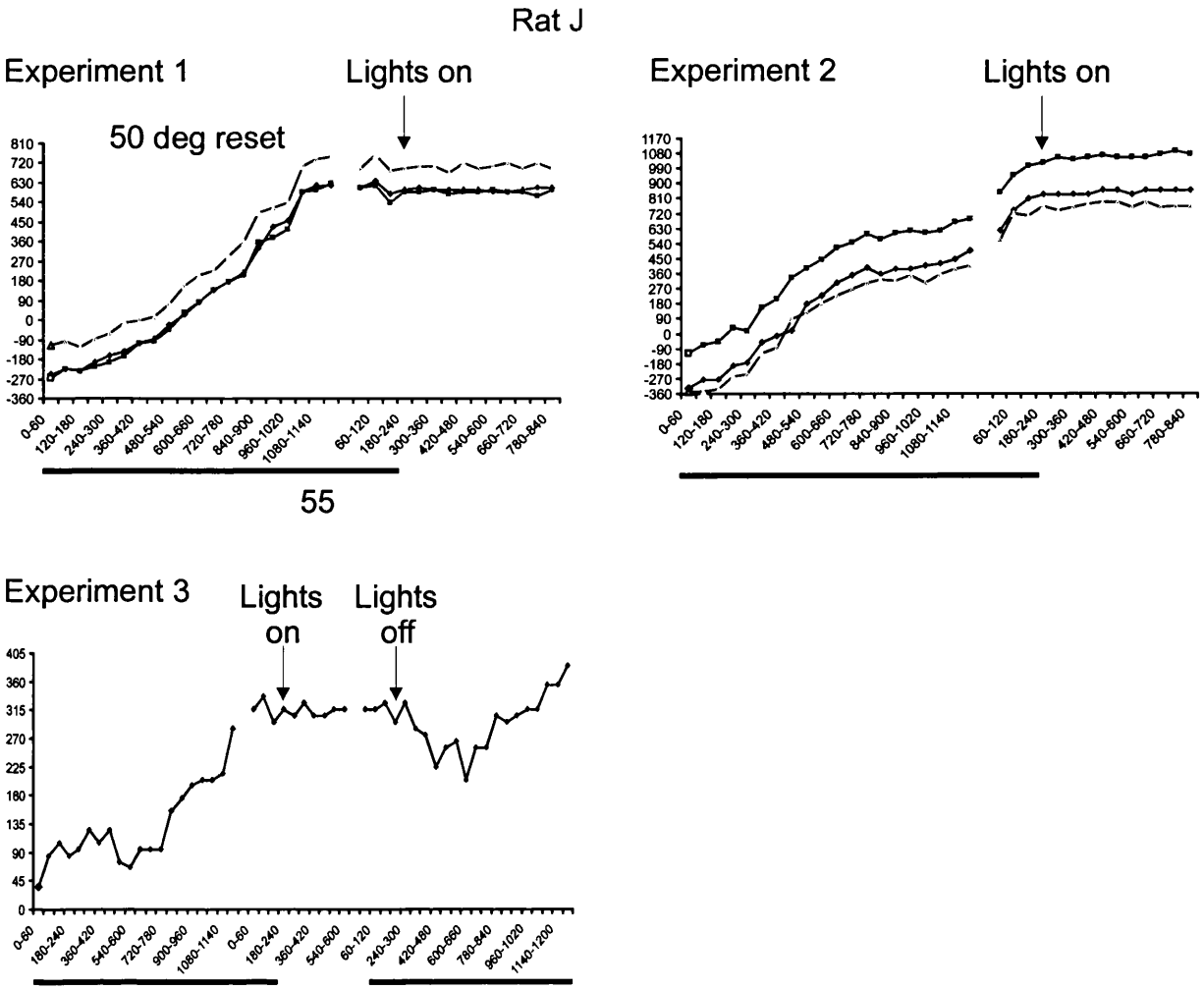
Rat H


Trial 1
Trial 2
0-60 60-120 120-180 180-240 240-300 300-360 360-420 420-480

B


Rat F
0-60 120-180 240-300 360-420 480-540 600-660 720-780 840-900 960-1020 1080-1140 1200-1260

C


Rat J
Experiment 1
Lights on
50 deg reset
55
Experiment 2
Lights on
Experiment 3
Lights on
Lights off

Rat E

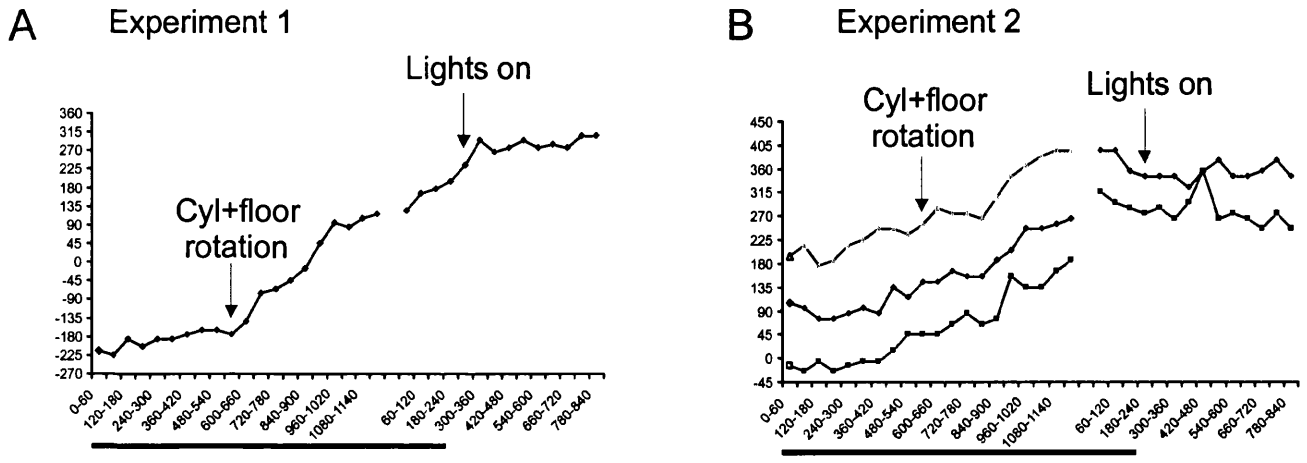


Fig XIV.7 Head direction cells responses in the dark.

Graphs show the values of preferred direction at one minute intervals. Black bars at the bottom of graphs indicate dark periods. Discontinuities in the preferred direction lines indicate the end of one trial and beginning of next trial.

Two experiments are shown (A and B), 24 days intervened between them. Each experiment consisted of two trials. The 1st trial was 20 minutes long, lights were off at all times. At 10 minutes during this trial the animal was lifted and the cylinder and floor rotated 180 degrees. The animal was left in the cylinder between the first and second trials. Trial 2 was 14 minutes long, during the first 4 minutes the lights were off, then were switched on for the remaining 10 minutes.

A) One HD cell was recorded. The cell's preferred direction was stable during the first 10 minutes of the first trial. The preferred direction rotated counterclockwise approx. 100 degrees after the cyl+floor rotation, indicating that the cell was using tactile/olfactory cues present on the cylinder and/or floor to update its directional preference. The cyl+floor rotation induced a drift in the cell's preferred direction at an average speed of 65 deg/min. This drift stopped only during the second trial, after the lights were turned on. The drift was probably induced by the fact that after the cyl+floor rotation, a conflict was introduced between idiotethic and sensory cues available to the HD system.

B) Three HD cells were recorded during experiment 2. One of the cells was lost (probably due to electrode drift) at the end of the first trial.
A very slow, counterclockwise drift is observable from the start of the first trial (average speed 4 deg/min). This time the preferred directions of the cells did not follow the cylinder + floor rotation. This induced an acceleration in the rate of drift (average 18 deg/min). At the beginning of the second trial the drift was slow and clockwise and the preferred directions "froze" when the lights were turned on.

separated from the other by one week and it consisted of 20 minutes recording in darkness, then recording was stopped for up to three minutes to allow for data transfer, and a new 14 min trial was started. Of these 14 minutes the first 4 were in darkness and the last 10 the lights were on. During the intertrial interval the animal was left in the dark within the cylinder. On day 1 three HD cells were recorded. Onset of the drift was almost immediate, and was counterclockwise, at a speed of 46°/min. When recording was started again, after the data transfer, the cells did not drift, and they kept being stable after the lights were switched on. Another interesting detail about this experiment is that upon entering the curtained enclosure, at the beginning of the 20 minutes trial, the cell's preferred directions were an average of 50° out of phase with the baseline values. This is the only instance in which the preferred direction of HD cells was not the same in the baseline (light) and darkness condition, and is very similar to what happened to cell HD f2 (see fig XIV.3).

On day 2 (seven days later) a very similar result was obtained: the preferred directions of the 3 HD cells recorded drifted counterclockwise from the very start of the trial at an average speed of 43°/min. This time the drifts extended to the 4 minutes darkness of the second trial and stopped abruptly when the lights were turned on. This time the preferred directions of the three HD cells recorded was the same in the baseline and at the beginning of the dark trial.

The experimental protocol for day 3 was somewhat different, and the reader is referred to the picture for greater detail. In this case the preferred directions showed a minor instability across the first 10 minutes of the dark trial and then started rotating (always counterclockwise) at 27°/min. The drift stopped when the lights were switched on and started again when they were switched off for the second time. These results suggest that the rat's directional system had learned, during the two preceding dark experiences, to cope with the absence of visual inputs, at least temporarily (ten minutes).

The results presented in fig XIV.7 relate to two experiments very similar to those described for cell TD f3 and TD f6. The cells were recorded for ten minutes in the dark, then the rat was lifted up and the cylinder+floor rotated by 180° (as a control, the rat was also picked up at 5 minutes during the trial, while the environment was kept still), and recording lasted for ten more minutes. The second trial was 14 minutes long (4 dark, 10 light), and the rat was kept inside the environment, in the dark, during the intertrial interval.

Rat E had extensive experience in the recording environment before the experiments presented took place, and had been exposed twice to a simple dark protocol, where he was left in the cylinder, in complete darkness for 20 minutes (data not shown). In the first case the difference in the preferred directions of the cells between the first and last minute of the trial was 40°; in the second experiment no drift was observed at all.

The experiments presented here were separated by 24 days and no intervening dark session was run between them. In experiment 1, during the first ten minutes of darkness the HD cell's preferred direction was rather stable (drifting very slowly at 4°/min), and started drifting counterclockwise at 65°/min after the cyl+floor rotation occurred. The drift extended into the second trial and the preferred direction of the

cell stabilised only one minute after the lights were turned on. This result is taken to suggest that either tactile or olfactory cues (if one assumes that darkness was truly depriving the animal of visual inputs) bound to the cylinder and/or the floor were allowing the directional system to maintain a stable orientation. The preferred direction of the HD cell shifted by 100° around the time the cyl+floor were rotated, indicating that perceptual features bound to these surfaces were controlling (albeit not completely) the HD system. The degree of under rotation can be explained on the basis of the conflict that the manipulation had introduced between the rat's path integration system and the perceptual inputs coming from the cylinder and floor (they ended up being 180° out of register).

During the second experiment, the preferred directions of the three HD cells recorded drifted again very slowly at 4°/min during the first ten minutes of the trial, and after the cyl+floor rotation occurred, the drift accelerated, with an average speed of 18°/min. During the second dark trial the preferred directions of the cells were more stable and froze completely once the lights were turned on.

In conclusion, all these dark experiments indicate that in the absence of visual inputs, the directional system is prone to instability. This effect is more pronounced in some animals, and repeated experience of the dark condition can induce a reduction in the amount of instability observed (see rat J experiment 3). In one animal (rat E), for which HD preferred directions were found to be constant during dark trials, instability could be induced by introducing a conflict between idiothetic and tactile/olfactory information.

Barriers

When physical barriers are introduced inside the recording environment, hippocampal place fields show varied responses: 1) if the barrier bisects or encroaches the field, a sharp suppression of firing is observed; 2) if the barrier is not placed within the cell's place field, usually no change in either shape or firing rate occurs, 3) new fields, of previously silent place cells emerge in close proximity of the barrier; 4) or a new sub-field of a previously recorded place cell is established, in close proximity to the barrier (Muller et al, 1987, Lever et al, 2002b).

The activity of six topodirectional cells was recorded before, during and after the insertion of physical barriers inside the recording environments (cylinder, square, or big square). Given that only a small group of cells was tested, and that the responses observed were heterogeneous a description of the results of each experiment performed is given below.

Fig XIV.8 shows the results of two experiments performed on subsequent days on cell g1. Each trial was 10 minutes long. In trial B, a wooden barrier, made of the same material and painted in the same colour as the cylinder, was introduced obliquely in the recording environment. The barrier insertion induced a shift in the cell's locational field, while leaving its directional field virtually unchanged. The locational field of the cell moved towards the barrier, flattening against it. The next day, the same barrier was introduced inside the square enclosure, parallel to its north and south walls. Trials A and B were 10 minutes long, while trial C was 15 minutes

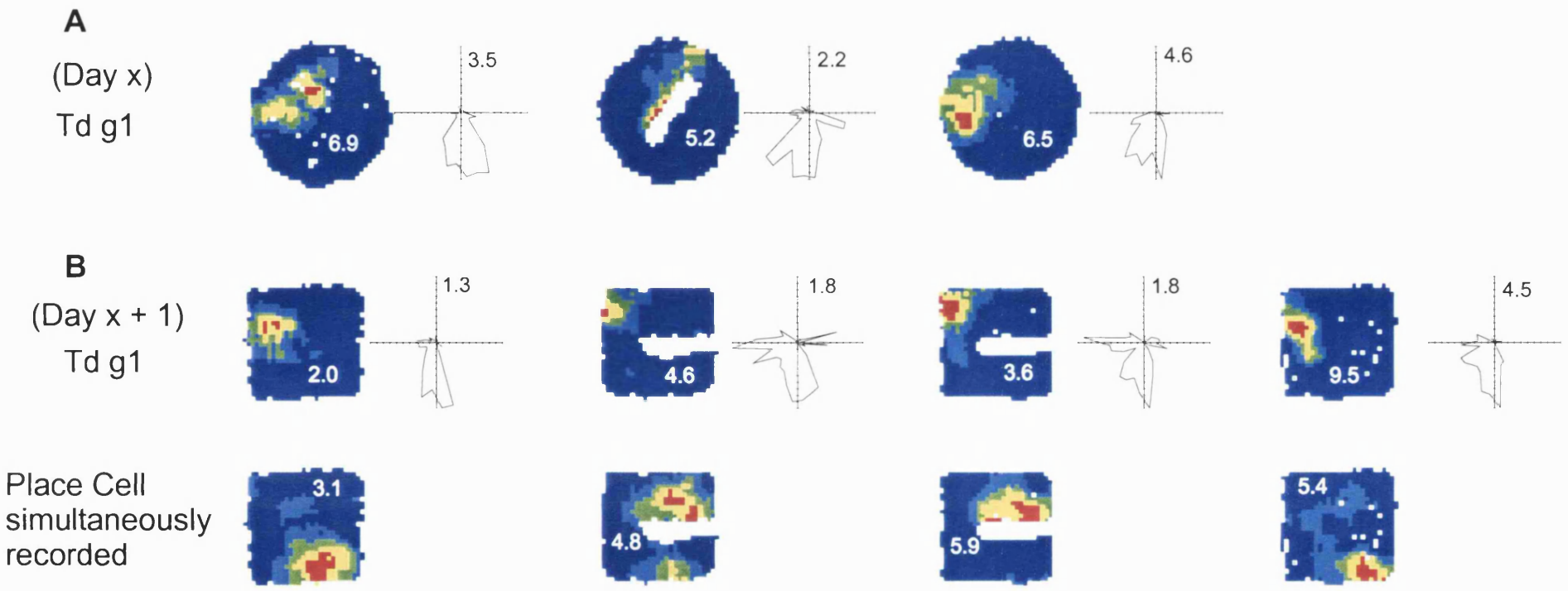


Fig XIV.8 A topodirectional cell (TD g1) was recorded on two successive days shown in A) and B). Trials are presented in temporal sequence. Note that the rat is *not* removed from the environment after the third trial in B);after nine minutes, the barrier is removed suddenly wihile the rat is in the square enclosure. The fourth trial of B) lasts six minutes.

In both cases the barrier induces a small but clear shift in the locational field of the cell. In the barrier condition in A) the field shifts closer to the barrier, in the B) barrier condition, the field shifts away from the barrier. There is not much change in the directional preference of the cell in A), but there is an additional western component to the directional firing in the B) barrier condition.

In the simultaneously recorded place cell, the barrier induces a doubling of the field, and then the original field dies out. This field is reinstated when the barrier is removed.

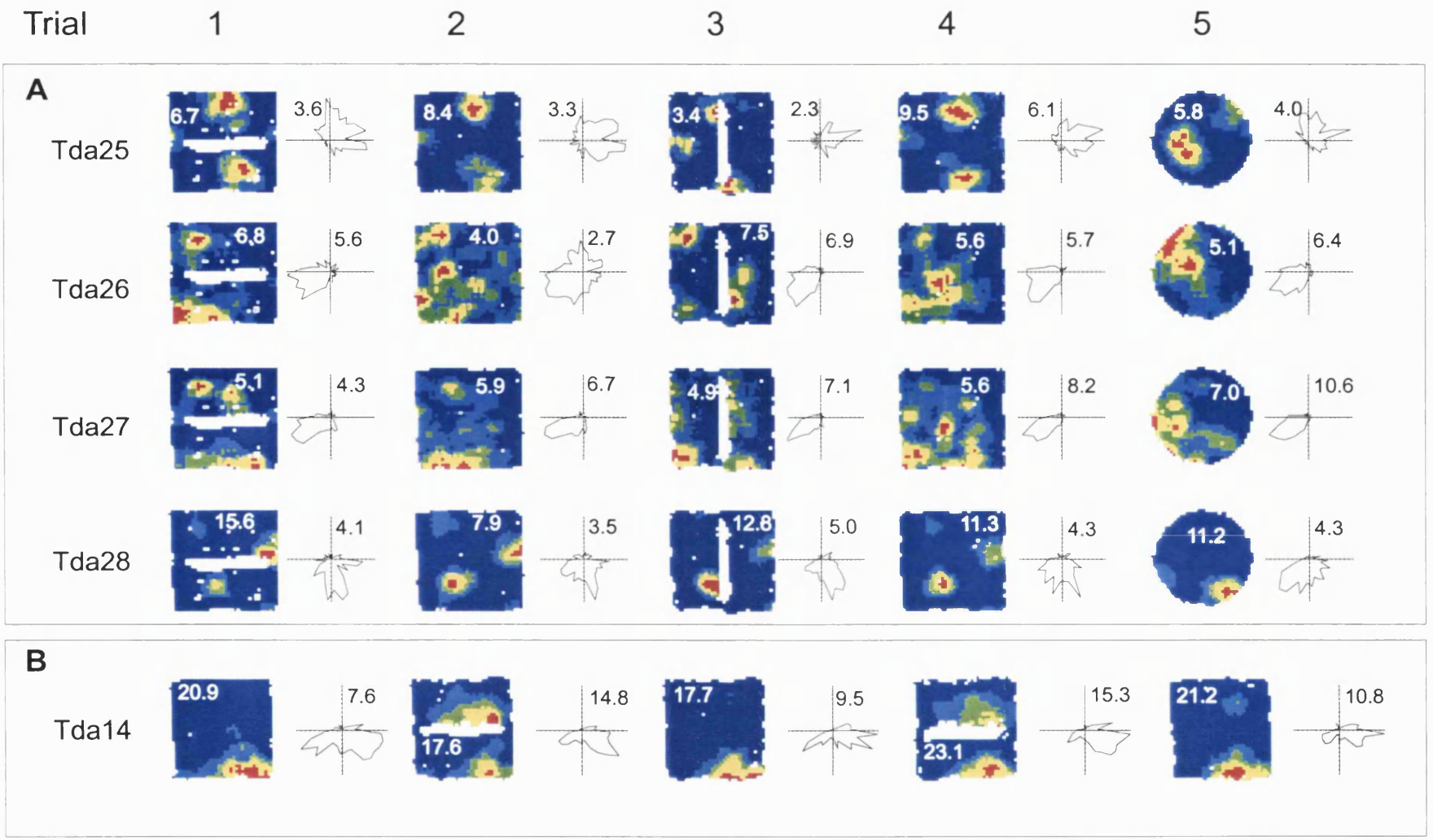


Fig XIV.9 Trials in A) and B) are taken from different recording days. Within each day, the trials are presented in temporal order. The four cells in A) were recorded simultaneously.

A) The barrier appears to cause in increase in in-field firing in cells Tda26 and Tda28, and a decrease in firing in cell Tda25. Cell Tda25 shows some evidence of locational field remapping. The directional firing of the cells does not really alter with the barrier condition, with the exception of the transitory effect in trial 2 in cell Tda26.

B) The barrier induces a second locational field in a position predicted by the model of Hartley et al (2000), as if the barrier were acting a a kind of south wall. Note that there is some after-effect of barrier condition in the fifth trial.

long: during the first 9 minutes the barrier was present in an identical position to trial B, and then it was lifted away from the square. Two pictures from the first 9 and last 6 minutes of trial C are separately shown in fig XIV.8B. In this experiment, cell TD g1 was simultaneously recorded with a place cell. The responses of the two cells are very different: the locational field of the topodirectional cell shifts north, away from the barrier and the west component of its directional field gets strengthened, while the place cell develops a new sub-field just north of the barrier, mirroring the original one north of the square's south wall. During trial C, the place cell stops firing in the original location and firing is only observed north of the barrier. This implies that barrier-induced changes are plastic. When the barrier is removed, during trial C, the topodirectional cell returns to baseline and the original field of the place cell is reinstated. Residual firing is observed in the region where the barrier induced sub-field was located. The topodirectional response to the introduction of the barrier in the square is reminiscent of some place cell responses described by Muller et al (1987). When the barrier was not directly located within the place cell's place field, sometimes its place field would just move slightly, away from the barrier. The place cell response in the experiment just described is more interesting, and could be explained by the computational model proposed by Hartley et al (2000) described in the introduction to this thesis. Briefly, the activity pattern of this cell in the barrier condition suggests that its firing is induced at a fixed distance (very short in this case) from a southerly placed wall, and that the barrier acts exactly as the south wall of the square. Even more interestingly, there is a suppression of the original firing field, the second time the animal is exposed to the barrier condition, implying that bimodal firing is only temporary, and not well tolerated within the place cell system.

Fig XIV.9B refers to a similar experiment conducted on a topodirectional cell (TD a14). This time the barrier was placed centrally inside the square enclosure, parallel to its north and south walls. This topodirectional cell behaves in a very similar manner to the place cell just mentioned. Upon the introduction of the barrier (trials 2 and 4), the locational field of the cell is doubled, with a new sub-field immediately emerging just north of the barrier. In the baseline conditions the sub-field is either not there (trial 1), or barely detectable (trials 3 and 5). Interestingly, there is an increase in the strength of the sub-field in trial 5 when compared to trial 3, suggesting that repeated exposures to the barrier condition "facilitate" topodirectional activity in the sub-field location, even in the absence of the barrier (see the last trial). The only difference with the results relating to the place cell, shown in fig XIV.8B, is that the original locational field of the topodirectional cell is not suppressed over repeated exposures to the barrier condition.

Fig XIV.9A shows the results of another experiment where 4 TD cells were simultaneously recorded from rat a. In two separate trials (1 and 3) one horizontal and one vertical barrier are introduced in the square environment. Cells' responses are heterogeneous. In general, directional fields are unaffected by the introduction of the barrier in either orientations. The position of the locational fields for cells a25 and a28 stays unchanged upon the barrier introduction, while those of cells a26 and a27 shift. It is unlikely that the locational fields of cells TDa25 and a28 are solely determined by extramaze cues, given that the firing pattern of these two cells is completely different in the cylindrical enclosure (trial 5). Thus one cannot conclude that the lack of effect of the barrier introduction is due to the cells being exclusively tuned to a set of perceptual stimuli outside the square enclosure.

During trial 3, the vertically placed barrier encroaches the two main locational fields of cell a 25 and a marked reduction in the cell's firing rate is observed. This effect is very similar to the one observed in place cells by Muller et al (1987). The responses of the other cells are harder to interpret. In the case of cell TDa28, during trial 1, when the east portion of the horizontal barrier is placed very close to the eastern locational field of the cell, the firing rate of the cell increases. This effect is opposite to that described for cell a25. For both cells a26 and a27 the introduction of the barrier (in either orientation) seems to induce a shift in the cells' locational fields away from the barriers, in a similar way to what observed for cell g1.

The fact that these varied responses were observed in simultaneously recorded cells shows that no global changes within the topodirectional system are brought about by the introduction of physical barriers in the recording environment. This is similar to that observed in the hippocampal place cell system.

In conclusion, the results of the barrier experiments described above, indicate, together with those of the previous chapter, that walls, and or physical barriers, are important determinants of TD cell locational firing. As in the case of geometric alterations of the recording environments (see previous chapter), no changes in the directional correlates of TD cells were observed upon the introduction of barriers (with the exception of cell TD g1, in fig XIV.8B).

The responses displayed by TD cells in the presence of barriers were heterogeneous, and similar to those reported for place cells (Muller et al, 1987; Lever et al, 2002). These results, together with those discussed in the previous chapter, suggest that the determinants of TD locational firing might be similar to those of place cells.

Discussion

Chapter XV

This thesis reports on a new category of cells, named topodirectional cells, which were recorded from the deep and superficial layers of presubiculum and the superficial layers of parasubiculum.
The firing of topodirectional cells is correlated to the animal location *and* head direction in an allocentric reference frame, and is powerfully modulated by the theta oscillation.
We will discuss:

1) the general properties of topodirectional cells within the framework of other cells recorded in the hippocampal-parahippocampal network which show similar spatial characteristics;
2) the possible anatomical origin of the locational and directional signals that converge onto topodirectional cells;
3) the possibility that topodirectional cells are an input to the hippocampal place cells;
4) the theta modulation observed in topodirectional firing;
5) the effects of environmental manipulations on topodirectional firing;
6) the possible functional roles of topodirectional cells in the hippocampal navigational system.

General properties of topodirectional cells

The hippocampal formation plays a critical role in spatial navigation (O'Keefe and Nadel, 1978). This has been established through a wealth of lesion/behavioural and electrophysiological studies.

At the electrophysiological level, two classes of cells have been identified within the hippocampal-parahippocampal network that present clear spatial firing correlates: place cells in the hippocampus proper (CA1 and CA3; O'Keefe and Dostrovsky, 1971) which code for the animal's location, and head direction cells in the presubiculum (and many other extrahippocampal areas) which code for the animal's head direction in the horizontal plane (Taube et al, 1990a,b).

Topodirectional cells combine the spatial correlates of hippocampal place cells (location) and presubicular head direction cells (direction). Cells which have locational and directional correlates have been described throughout the hippocampal-parahippocampal network, though only pre- and parasubiculum contain cells whose firing is strongly modulated by both. We will begin by reviewing the nature of spatial firing in these brain areas. They are: subiculum (Sharp and Green, 1994), parasubiculum (Taube, 1995c), restrosplenial cortex (Chen et al, 1994; Cho and Sharp, 2001), and presubiculum (Taube et al, 1990a; Sharp, 1996).

In the case of subicular cells, location is the principal correlate, and only a subset of the recorded cells (60%) showed a secondary directional correlate. The strength of directional modulation was modest, with direction accounting for only 13% of the firing rate variance. Even by visual inspection, the directional modulation of a subicular cell which was classified as "relatively strongly modulated by directional heading" (Sharp and Green, 1994; fig 4), appears much weaker than that of any topodirectional cell. Similar considerations apply to parasubicular "place" cells (here place is in brackets to distinguish these cells from hippocampal place cells). Out of the16 "place" cells recorded only 3 showed a secondary directional firing correlate (Taube, 1995c).

Of the cells recorded in the restrosplenial cortex (Cho and Sharp, 2001), 20% were classified as "direction-dependent place cells". All the cells which were included in this category showed complex responses to combinations of specific locations and head directions. For example, one of the cells described fired in the centre of the cylinder only when the closest wall of the cylinder was to the left and behind the animal's head (Cho and Sharp, 2001, fig 5). The directional tuning curves of these retrosplenial cells always show several peaks. This complex directional response is in no way similar to that of topodirectional or any other directional cell recorded so far. Moreover only 2 out of the 23 cells included in the direction-dependent place cells category showed theta modulation.

The only cells which combine locational and directional correlates in a similar way to topodirectional cells were recorded by Taube (1995c) in the parasubiculum and by Sharp (1996) in the presubiculum. The latter represented roughly 20% of the cells recorded in that study and were classified as "place-by-head direction" cells. Only 2 out of the 14 cells which fell into this category showed theta modulation of their firing, in a similar way to the topodirectional cells presented in this thesis.

Thus, topodirectional cells in pre- and parasubiculum, together with the place-by-head direction cells in presubiculum, are the only cells which truly combine the two major spatial correlates described in the hippocampal-parahippocampal network: place and head direction. It is interesting that the place-by-head direction cells and most of the topodirectional cells were recorded from the presubiculum, suggesting that this structure might be more than just a channel through which the head direction signal enters the hippocampus.

Moreover, the powerful theta modulation observed in topodirectional cells (and which distinguishes them from the place-by-head direction cells), could have an important functional role, placing them within the wider theta network that is currently being discovered in many brain areas (hippocampal-parahippocampal network and areas traditionally included in the Papez circuit).

Possible anatomical origin of the locational and directional signals of topodirectional cells: anatomical and functional considerations.

Topodirectional cells lie at the crossroads between place and head direction cells. That is why, throughout this thesis, the characteristics of topodirectional cells have been closely compared to those of hippocampal place cells and presubicular head direction cells. This comparison has led to the general conclusion that while topodirectional cells show, for a variety of spatial measures, intermediate values between those of place and head direction cells, their directional firing seems to be more robust and powerful than their locational firing. Moreover, the directional firing of topodirectional cells appears to be closer to that of head direction cells than their locational firing is to that of hippocampal place cells. This was found to be true for both the uncorrected and corrected data, excluding the possibility that the deeper directional modulation of topodirectional firing is artefactual, resulting from inhomogeneities of sampling discussed in detail in chapter X. This is an important consideration, given that the majority of topodirectional locational fields are close to the edge of the cylinder, and the sampling bias due to the "edge effect" might have added spurious directional modulation to topodirectional firing. Even when these artefacts are removed, using the PxD algorithm, the relative strength of the directional and locational components of topodirectional firing remains unchanged: head direction appears to be a stronger determinant of firing than location.

In this section we will discuss the possible anatomical and functional sources of both the directional and locational correlates shown by topodirectional cells.

It is very likely that topodirectional cells receive directional inputs from head direction cells. Anatomically, they are ideally located to receive direct inputs from head direction cells in several brain structures: presubiculum, lateral dorsal (LDN) and antero-dorsal (ADN) nuclei of the thalamus, and retrosplenial cortex. All these structures send direct projections to both the deep and superficial layers of pre- and parasubiculum, where topodirectional cells have been found (van Groen and Wyss, 1990a,b; Shibata, 1993; see anatomical introduction, chapter I). However, even if it is likely that the TD directional signal comes from presubicular head direction cells, no functional evidence for this has been presented in this thesis. A possible way to address this question would be to perform a time-shift analysis on the directional component of TD firing, given that head direction cells from presubiculum have been shown to code for the animal's current head direction (Blair and Sharp; 1995; Taube and Muller, 1998), while HD cells in both retrosplenial cortex and ADN anticipate the rat's head orientation, on average, of 25 ms (Cho and Sharp, 2001; Blair and Sharp; 1995; Blair et al, 1997; Taube and Muller, 1998).

The origin of topodirectional locational firing is less clear. As discussed in the anatomical introduction to this thesis (chapter I), pre- and parasubiculum can be thought of as an output from, as well as an input to, the hippocampus proper. It is therefore possible that TD locational firing derives (either directly or by way of the subiculum) from that of hippocampal place cells. Another possibility is that their

locational signal derives from extrahippocampal inputs to pre- and parasubiculum. We will examine these two possibilities separately, using both the anatomical and functional evidence available.

The anatomical location of only a subset of topodirectional cells has been established with reasonable certainty (see Histology section, chapter VII). In these cases, topodirectional cells were localised in the superficial layers of the pre- and parasubiculum (animal D, two cells in two hemispheres), and in both the superficial and deep layers of presubiculum (in animal F, 7 topodirectional cells).

Anatomically, both superficial and deep layers of pre- and parasubiculum may receive significant locational inputs directly from CA1, or indirectly from CA1, through subiculum. Projections from CA1 to pre- and parasubiculum are rather sparse and contact both the deep and superficial layers of these structures (Van Groen and Wyss, 1990b), while subicular projections are denser and terminate in both superficial (I) and deep (V) presubicular layers, and layers I and II in parasubiculum (Kohler, 1985; Van Groen and Wyss, 1990a,b).

An experiment which might clarify if topodirectional cells are an output of the hippocampus proper would be one where the hippocampus is lesioned or temporarily inactivated and topodirectional cells recorded. Would topodirectional directional properties remain, while their locational properties disappear? Or rather would they stay unchanged, proving that hippocampal locational input is not necessary in the maintenance of topodirectional place-like signal?

An alternative view is that TD locational characteristics derive from extrahippocampal inputs to pre- and parasubiculum. These are mainly visual in nature, originating in retrosplenial cortex (Van Groen and Wyss; 1990a,b) and area 18b (Vogt and Miller, 1983). Retrosplenial projections mainly originate in layer V and target both superficial and deep presubicular layers (layers I and III-V; Van Groen and Wyss; 1990a,b), while direct projections from area 18b target only the superficial layers of this structure (layers I and III; Vogt and Miller, 1983). At the functional level we know that visual information plays a crucial role in the maintenance of topodirectional firing. When recorded in total darkness, a strong reduction or a total suppression of firing was observed in 2 out of 3 cells examined (see chapter XIII). This result is interesting, given that neither hippocampal place cells nor head direction cells show a decrease in firing rate when recorded in darkness (Taube, 1998, Quirk et al, 1990). The peculiar sensitivity of topodirectional cells to the absence of visual information could well reflect their anatomical location, and indicates that, a least in some cases, path integration and sensory cues other than visual are not able to support topodirectional firing. It also shows that topodirectional cells are not merely reflecting inputs received from head direction and hippocampal place cells.

Is the topodirectional system an input to the hippocampal place system?

The previous section discussed the hypothesis that topodirectional locational properties might derive from those of hippocampal place cells. An important and related issue is whether topodirectional cells could act as an input to the hippocampus proper. We will discuss this possibility after briefly reconsidering the possible nature of the TD signal recorded.

As exposed in the discussion section of chapter VIII, it is impossible to know if the source of the TD signal recorded were fibres, interneurons or principal cells. This is because no clear relationship has been found between parameters like the extracellularly recorded waveform shape, signal polarity and amplitude and cytological identity. Therefore we concluded that TD signals have more likely been recorded from a heterogeneous population which may contain both interneurons (in the case of the small amplitude, tri-phasic waveforms) and principal cells (bigger amplitude, pyramidal-like waveforms) in both the pre- and parasubiculum. We could not exclude that in some cases TD signals were generated by fibres.

Given that some of the TD cells recorded might have been projecting cells, in the following section we will consider the possibility that TD cells could act as input to the hippocampal place cell system. The route of topodirectional signals into the hippocampus proper (CA1 and CA3), could be through pre- and parasubicular projections to the superficial layers (III/I and II, respectively) of entorhinal cortex (Kohler, 1985; Witter et al, 1988). More specifically, as superficial layer cells in both pre- and parasubiculum densely innervate entorhinal-to-hippocampal projection neurons (Cabellero-Bleda and Witter, 1994) it is anatomically plausible that topodirectional cells provide disynaptic input to place cells.

A relevant physiological result is that we recorded one topodirectional unit (TD g1) from a region just below CA1 stratum pyramidale. This result suggests that topodirectional signal reaches the hippocampal formation. TD g1 was simultaneously recorded with a hippocampal place cell. Its waveform was inverted, the amplitude very big (reaching a maximum of 1 mV), and the signal was picked up from only one of the four tetrode wires. This suggests that the electrode was probably juxtaposed to a cell/fibre membrane; curiously, we could record this unit over two days, suggesting that the electrode-cell/fibre arrangement was somewhat stable.

TD g1 anatomical location makes it unlikely that the source of TD firing was a fibre of passage (which does not make contact with hippocampal neurons), while leaving open two possibilities, TD g1 could have been: 1) a local hippocampal interneuron or 2) an afferent fiber to the hippocampus proper. It is impossible to distinguish between these two possibilities on the basis of the available evidence.

The only functional study that might shed light on the issue of whether topodirectional cells act as an input to the hippocampus proper has been unfortunately published only in abstract form (Archey et al, 1997). The authors of this study lesioned the presubiculum (and in a separate set of animals the ADN) and recorded

hippocampal place cells. Surprisingly, place cells recorded from animals in both lesion groups showed an increase in directionality, and lack of cue control. One possible interpretation of these results is that the absence of cue control was determined by the ablation of directional input (from head direction cells) to the place system, and that the increased directionality seen in place cells could be due to a partial lesion in the topodirectional system. Given that parasubiculum was largely intact (Taube, pers. comm.), place cells in the lesioned animals could receive only a portion of topodirectional inputs and this might have produced place cells which are more like topodirectional cells. The assumption here is that that each place cell results from the summation of inputs of several topodirectional cells with spatially superimposable locational fields, and different directional preferences. The fact that ADN lesions produced similar effects on CA1 place cells as presubicular lesions might be explained on the basis that TD signal was probably disrupted in these animals, given that TD directional inputs most likely derive from those of HD cells.

In conclusion, a bisynaptic anatomical connection exists, in both directions, between pre-/parasubiculum, and CA1, and it is possible that topodirectional cells are feeding into and/or receiving locational inputs from place cells. Topodirectional cells could therefore form part of an extended hippocampal system loop. It is still unclear, and of crucial functional importance, to which extent topodirectional locational correlates are the product of intra- vs extrahippocampal inputs. This might be tested by recording TD cells before, during and after reversible hippocampal inactivation.

Theta modulation

Topodirectional firing is deeply modulated by theta. The degree of theta modulation in TD cells is comparable to that seen in septal cells, which are thought to drive theta oscillation in the hippocampus (see fig XII.5).

Theta can be recorded as a field oscillation throughout the hippocampal formation, pre- and parasubiculum and entorhinal cortex. At the cellular level, theta modulated units have been recorded in the freely behaving rat, in many brain regions, but they generally represent only a small proportion of the total cells recorded: 12% in the subiculum (Sharp and Green, 1994), between 15 and 17% in the presubiculum (Taube et al, 1990a; Sharp, 1996), 42% in parasubiculum (Taube, 1995c). Theta modulated units have also been reported in both the superficial and deep layers of entorhinal cortex (Frank et al, 2000, 2001), in both the granular (60%) and agranular (27%) regions of the retrosplenial cortex (Cho and Sharp, 2001), in the anterior nucleus of the thalamus (AD, 1%; Taube, 1995a) and the lateral mammillary nuclei (4.6%, Stackman and Taube, 1998).

In the urethane anaesthetised rat theta modulated cells have also been found in the anterior ventral (AV) nucleus of the thalamus (where up to 75% of the recorded cells are theta modulated with 46% of these being strongly modulated by theta, Vertes et al, 2001).

Presubicular topodirectional cells could receive theta modulation from both the septal nuclei, or the AV, directly or by way of retrosplenial cortex.
At the functional level, the deep theta modulation of TD cells sets them apart from the place-by-direction cells recorded by Sharp (1996) in the presubiculum, which have similar spatial correlates to topodirectional cells. TD cells might be part of a wider theta network which encompasses brain areas in both the hippocampal-parahippocampal network (hippocampus proper, subiculum pre- and parasubiculum, entorhinal cortex) and areas which belong to the Papez circuit (anteroventral nucleus of the thalamus, lateral mammillary bodies, restrosplenial cortex).

Topodirectional cells recorded from both the superficial and deep layers of presubiculum are strongly modulated by theta. Similarly, within the entorhinal cortex both superficial and deep layers cells were theta modulated (Frank et al 2000, 2001). These results are at odds with the two-stage model of memory formation, put forward by Buzsaki (1989; see chapter II, p.19), which relies on the idea that while cells in superficial layers of parahippocampal regions are entrained in the theta oscillation (Chrobak and Buszaki, 1994), cells in the deep layers of these structures show increased firing during hippocampal sharp waves (Chrobak and Buszaki, 1994), and fire preferentially at the negative peaks of the locally recorded ripples (Chrobak and Buszaki, 1996), while being insensitive to theta (Chrobak and Buszaki, 1994).

Topodirectional cells fire preferentially at the negative-to-positive phase of the locally recorded theta oscillation. In this, they are remarkably similar to interneurons recorded from the stratum pyramidale in region CA1 (Chrobak and Buzsaki, 1999). TD phase preference is quite tight (spikes are strongly clustered around the preferred theta phase), suggesting that TD cells do not show phase shift, unlike place cells (O'Keefe and Recce, 1993).

Effects of environmental manipulations on topodirectional firing

The effects of environmental manipulations on topodirectional firing will be discussed with reference to results of similar manipulations conducted on place and head direction cells.

We have shown that the directional preference of TD cells is stable across recording sessions and constant across environments which do not share obvious sensory cues (the holding platform outside and the cylinder inside the curtained enclosure). These results are in line with those reported for head direction cells (Taube, 1998) and show that TD cells code for the animal's head direction in an allocentric framework.
The cue control experiment run on two simultaneously recorded TD cells showed that the cue card is an important determinant of firing pattern in these cells, similarly to what reported for both place and head direction cells (Muller and Kubie, 1987; Taube, 1998). No uncoupling was observed between either the directional and locational fields within each cell, or across the spatial fields of the two cells. The two cells rotated in unison, similarly to what is routinely observed for head direction cells.

We also tested TD cells in differently shaped environments. Changing the shape of the recording environment did not significantly alter the directional preferences of the cells, which remained stable across the three environments tested: cylinder, square and open platform. These results might seem slightly at odds with those presented in the Taube et al (1990b) study, where the authors found that in several occasions the directional preferences of presubicular head direction cells changed across a cylindrical and a square-walled environment. We have already addressed this issue in the discussion session in chapter XIII, but it might be useful to remind the reader that in the Taube et al (1990b) study the animals were disoriented before being introduced in the curtained enclosure, while in all our experiments rats were never purposefully disoriented. Under our experimental conditions we never saw a difference in the preferred directions of head direction (data not shown) or topodirectional cells recorded in the cylinder and the square. We suggest that only in the absence of reliable path integration information, environmental geometry affects directional preferences of head direction and topodirectional cells.

The effects of environmental geometric alterations on TD locational firing are similar to those observed in hippocampal place cells which were recorded in this laboratory, under very similar experimental conditions (Lever et al, 2002). In general, TD locational firing patterns were similar across the cylinder and square, and changed ("remapped") between the cylinder and the open platform (a boundless circular platform). Place cells, recorded from naïve animals, show a similar pattern of results: place fields are similar across cylinder and square and remap between cylinder and open platform.

An important difference between topodirectional and hippocampal remapping, is that while topodirectional cells fire in all tested environments, it is not unusual for a place cell to fire only in a subset of the tested environments. For example, one place cell might fire in the cylinder and not in the open platform, or vice versa, while topodirectional cells always fire in both environments. This is also true of the firing patterns recorded from the holding platform (outside the curtains) and the cylinder (inside the curtains). Topodirectional cells always fire in both conditions (and remap, data not shown), while it is very common to find that place cells with fields on the holding platform will not have fields inside the curtained enclosure (and vice versa). The only instance when topodirectional cells stop firing is in darkness, unlike what happens to place cells (Quirk et al, 1990). The differences discussed here show that topodirectional firing cannot be thought of as a simple reflection of hippocampal locational firing.

In addition to topodirectional and place cells, subicular (Sharp and Green ,1994; Sharp, 1997; Sharp et al, 1999b) and medial entorhinal neurons (Quirk et al, 1992) have been tested in cylinder and square. Again, in both cases, firing patterns were similar across these two environments, showing that spatial representations of square and cylinder environments are similar across the whole hippocampal spatial network (but note that parasubicular "place" cells have not been tested in the square; Taube et al, 1995).

An important contribution of Lever et al (2002) has been to show that over subsequent exposures to square and cylinder, the hippocampal representations of

these two environments diverge. This phenomenon has not been observed in topodirectional, subicular (Sharp, 1997) and entorhinal cells (Quirk et al, 1992).

In the case of topodirectional cells, the animals never received a similar amount of experience to that required for the hippocampal place cells to show remapping. It therefore remains an open question, whether topodirectional locational fields would remap given enough experience, and how hippocampal and topodirectional learning curves would relate to each other: would learning be faster in the hippocampus or in the topodirectional system?

Very similar considerations apply to both subicular and entorhinal neurons. The absence of remapping in these two structures has traditionally been interpreted as proving that subicular and entorhinal cells participate in the generation of universal maps, as opposed to hippocampal maps, which were thought to differ between square and cylinder. Now we know that the difference seen in the hippocampal representations of cylinder and square might have reflected the animal's training history, given that experience was not controlled in all these remapping experiments (see chapter XIII, discussion).

It would be extremely helpful to perform experiments where hippocampal cells would be recorded simultaneously with either subicular, entorhinal or topodirectional cells, in both cylinder and square, over subsequent days. This type of experiment, though technically extremely challenging, would help understanding the nature of the functional interactions existing between different processing stages within the hippocampal navigational system.

Walls as determinants of topodirectional firing

The fact that topodirectional cells remap between the cylinder and the open platform suggests that walls are an important determinant of TD locational firing. These results are also complemented by those of the barrier experiments presented in chapter XIII. Briefly, when barriers are introduced in the recording environment, while TD directional preferences are usually unaffected, locational fields shift either towards or away from the barrier, and a reduction in firing is observed if the barrier bisects or encroaches the field. These results are very similar to those observed in hippocampal place cells (Muller et al, 1987, Lever et al, 2002b).

A particularly unambiguous result is that obtained for cell TD a14. This cell had a locational field right against the south wall of the square. Upon introduction of a barrier in the centre of the square and parallel to the south wall, a second field emerged immediately, on top of the barrier. This is a result which has been observed in place cells (this thesis and Lever et al, 2002b), and suggests that the barrier here is treated as a second "south wall", in line with the predictions of the model by Hartley et al (2000).

In many cases, the results of barrier experiments are not as easily interpretable (see TD a25-28). In general, it is not clear how to predict the locational firing of TD

cells between geometrically different environments (like the cylinder and open platform).

The fact that in general TD locational fields are close to the edge of walled environments tempts us to speculate that TD cells are coding for distance from physical barriers in the preferred direction of the cell. More specifically, TD cells might act as "barrier-detectors", given that many of the locational fields of TD cells are clustered close to the wall of the environment. No experiments have been performed that could formally test this hypothesis.

Functional role of topodirectional cells

We have already stressed that pre- and parasubiculum occupy, anatomically, a central position within the hippocampal-parahippocampal network. They can be seen both as input and output to the hippocampus proper as well as taking part in a re-entrant loop, re-channelling information already processed by the hippocampus via the entorhinal cortex, back into the hippocampus proper. It is therefore interesting that pre- and parasubiculum are the only brain regions within the hippocampal circuit that contain cells which code for both location *and* head direction. We will discuss the possible functional role(s) that topodirectional cells have within the hippocampal navigational system. In particular we will refer to navigational models of hippocampal functioning that have explicitly advocated the presence of cells with similar spatial correlates to those observed in topodirectional cells. We will also discuss the possibility, already mentioned in an earlier section, that topodirectional cells could function as building blocks of the hippocampal place signal.

Sharp et al (1995) proposed a model of hippocampal functioning which we have reviewed in the introductory section of this thesis (chapter V, p.54). This is a stimulus-response model and predicts the presence of place-by-head direction cells, placing them in the nucleus accumbens. These cells represent an interface between the hippocampal navigational system and motor regions. Reward history sets up asymmetric weights in the connections between place-by-head direction units and motor efferent cells (left and right turn cells). In this context, topodirectional cells would lie at the output end of the hippocampal formation, providing a bridge between the hippocampal navigational system and motor structures. Even though stimulus-response models suffer from being too inflexible, it is conceivable that over-trained routes could be encoded, outside the hippocampus proper, through an interaction between place-by-direction cells and motor structures.

Several authors have modelled the hippocampal place representation as a bidimensional neural attractor (McNaughton et al, 1996; Sharp, 1999c; Samsonovich and McNaughton, 1997). These models share the central idea that any given environment will be represented through a set of hippocampal place cells, which are linked together so that cells which fire in contiguous places are strongly interconnected by excitatory synapses, while place cells with fields in widely separated positions, are only weakly interconnected. A global inhibition mechanism guarantees that only one set of strongly interconnected place cells can be active at any given time (corresponding to a specific position in the environment).

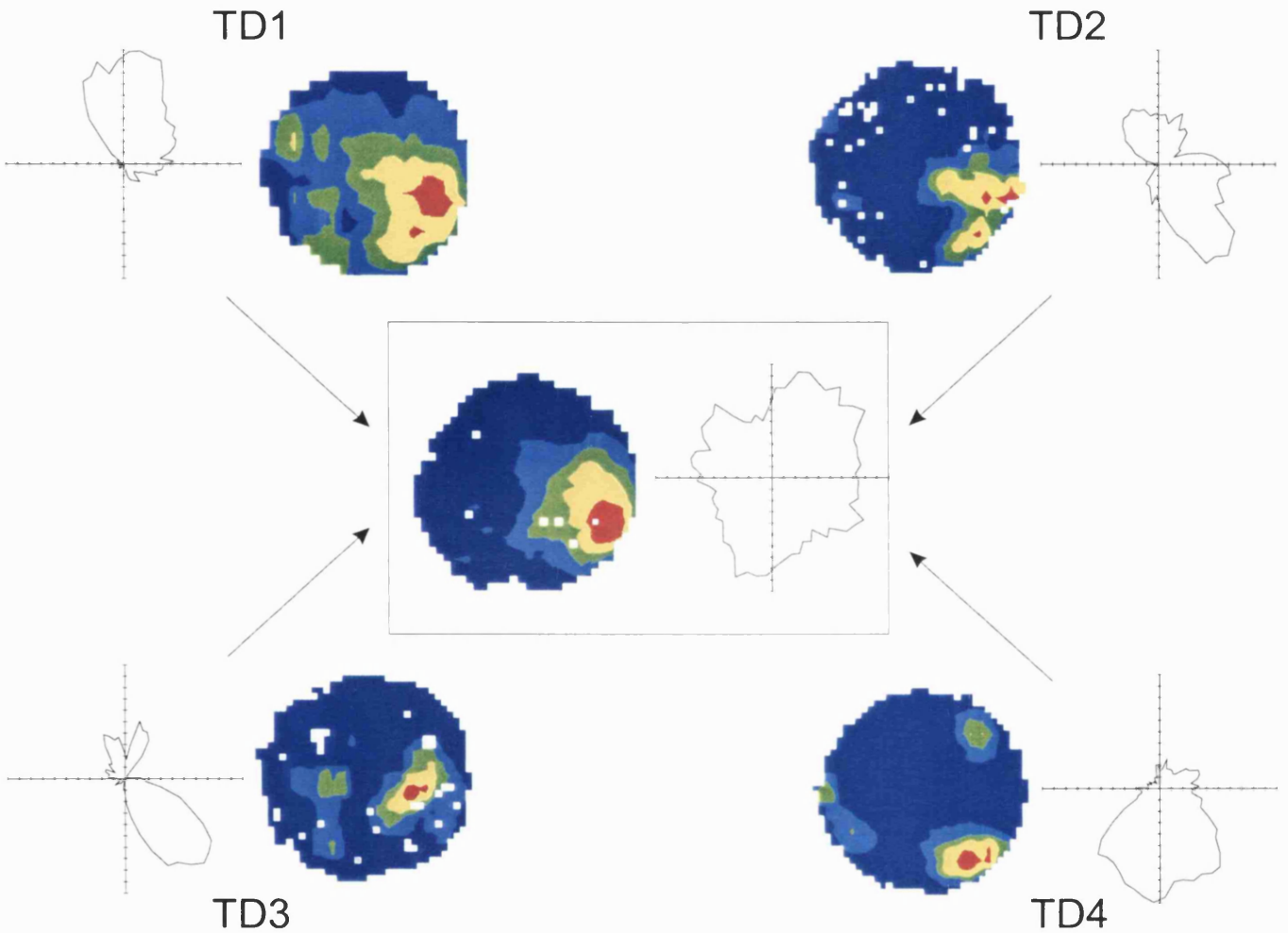


Fig XV.1 This diagram uses real data from 4 topodirectional (TD) cells (at corners of diagram) and 1 place (PC) cell (in centre of diagram), to present a hypothetical account of how topodirectional inputs could sum to provide an omnidirectional locational signal.
The place cell fires in the south-eastern portion of the cylinder. Note that the place cell fires roughly equally in all directions faced by the rat within the place field.
All the topodirectional cells show peak firing in the south-eastern portion of the cylinder. The directional preference of each topodirectional cell is different and quite selective (eg. north for TD1, broad south for TD4) but together, all directions are represented. A hypothetical cell which simply summated inputs from all four cells would be likely to chow a locational field in the south-east region of the cylinder, regardless of the direction faced by the animal.

This architecture is similar to that used to model the head direction system (Skaggs et al, 1995; Redish et al, 1996; Sharp et al, 2001a).

An important element of all these models is that which is responsible for shifting the activity "hill" of the attractor, whenever the animal moves in the environment, changing head direction and position.

In head direction models, this function is performed by cells which receive their inputs from both head angular velocity and head direction cells (Skaggs et al, 1995; Sharp et al, 2001a), and which have asymmetric connections to the head direction cells. In the place cell models, it is performed by a path integrator system (PI), constituted by cells which receive inputs from place cells, head direction cells and motion detector cells. These cells are thought then to form asymmetric connections with hippocampal place cells. The proposed locus of the place-by-direction-by-movement cells is the subiculum (McNaughton et al, 1996; Sharp, 1997, 1999c). This is because subicular cells show a strong locational signal coupled with a secondary (weak) directional signal. Another possible anatomical location for these cells are the pre- and parasubiculum (Samsonovich and McNaughton, 1997). We have seen that both these structures contain cells which code for place and head direction (Sharp, 1996; and this thesis). Moreover cells which code for the animal's speed have been localised in the presubiculum (Sharp, 1996; Lever et al, in preparation). Anatomically, pre- and parasubiculum are ideally suited to channel this information back into the place cell system, through their extensive connections with the superficial layers of entorhinal cortex. In a slightly different model of path integration, Redish (1999) proposed that the subiculum-parasubiculum-entorhinal cortex represent an attractor network which supports path integration.

Though topodirectional cells have very similar properties to those described for the Integrator cells in the models here described, the main problem is that visual inputs seem to be crucial for the maintenance of topodirectional signal. This is in conflict with the idea that topodirectional cells could be one of the key elements in a path integrator mechanism.

We propose an alternative function of topodirectional cells. It is possible that topodirectional cells represent building blocks of hippocampal place cells. In a perhaps too simplistic model, several topodirectional cells, with different head direction preferences and similar locational fields would converge in the hippocampus proper, to generate any given place cell signal (see fig XV.1). This model is consistent with findings showing that lesions of presubiculum or ADN make hippocampal place cells more (not less) directional (Archey et al, 1997).

Bibliography

Alonso A, Kohler C (1984) A study of the reciprocal connections between the septum and the entorhinal area using anterograde and retrograde axonal transport methods in the rat brain. J Comp Neurol 225: 327-343.

Amaral DG, Dolorfo C, Alvarez-Royo P (1991) Organization of CA1 projections to the subiculum: a PHA-L analysis in the rat. Hippocampus 1: 415-435.

Amaral DG, Witter MP (1995) Hippocampal formation. In: G.Paxinos (Ed.) The rat nervous system. San Diego, Academic.

Archey WB, Stackman RW, Goodridge JP, Dudchenko PA, Taube JS (1997) Place cells show directionality in an open field following lesions of the head direction system. Soc Neurosci Abstr 23: 504.

Bannerman DM, Deacon RM, Offen S, Friswell J, Grubb M, Rawlins JN (2002) Double dissociation of function within the hippocampus: spatial memory and hyponeophagia. Behav Neurosci 116: 884-901.

Barnes CA, McNaughton BL, Mizumori SJ, Leonard BW, Lin LH (1990) Comparison of spatial and temporal characteristics of neuronal activity in sequential stages of hippocampal processing. Prog Brain Res 83: 287-300.

Bassett JP, Taube JS (2001) Neural correlates for angular head velocity in the rat dorsal tegmental nucleus. J Neurosci 21: 5740-5751.

Blair HT, Sharp PE (1995) Anticipatory head direction signals in anterior thalamus: evidence for a thalamocortical circuit that integrates angular head motion to compute head direction. J Neurosci 15: 6260-6270.

Blair HT, Sharp PE (1996) Visual and vestibular influences on head-direction cells in the anterior thalamus of the rat. Behav Neurosci 110: 643-660.

Blair HT, Lipscomb BW, Sharp PE (1997) Anticipatory time intervals of head-direction cells in the anterior thalamus of the rat: implications for path integration in the head-direction circuit. J Neurophysiol 78: 145-159.

Blair HT, Cho J, Sharp PE (1998) Role of the lateral mammillary nucleus in the rat head direction circuit: a combined single unit recording and lesion study. Neuron 21: 1387-1397.

Blair HT, Cho J, Sharp PE (1999) The anterior thalamic head-direction signal is abolished by bilateral but not unilateral lesions of the lateral mammillary nucleus. J Neurosci 19: 6673-6683.

Bland BH, Anderson P, Ganes T (1975) Two generators of hippocampal theta activity in rabbits. Brain Res 94: 199-218.

Bland BH, Colom LV, Konopacki J, Roth SH (1988) Intracellular records of carbachol-induced theta rhythm in hippocampal slices. Brain Res 447: 364-368.

Bland BH, Colom LV, Ford RD (1990) Responses of septal θ-on and θ-off cells to activation of dorsomedial-posterior hypothalamic region. Brain Res Bull 24: 71-79.

Bostock E, Muller RU, Kubie JL (1991) Experience-dependent modifications of hippocampal place cell firing. Hippocampus 1: 193-205.

Bragin A, Jando G, Nadasdy Z, Hetke J, Wise K, Buzsaki G (1995) Gamma (40-100 Hz) oscillation in the hippocampus of the behaving rat. J Neurosci 15: 47-60.

Bragin A, Engel J, Wilson CL, Fried I, Buzsaki G (1999) High-frequency oscillations in human brain. Hippocampus 9: 137-142.

Brankack J, Stewart M, Fox SE (1993) Current source density analysis of the hippocampal theta rhythm: associated sustained potentials and candidate synaptic generators. Brain Res 615: 310-327.

Brazhnik ES, Fox SE (1997) Intracellular recordings from medial septal neurons during hippocampal theta rhythm. Exp Brain Res 114: 442-453.

Brown MA, Sharp PE (1995) Simulation of spatial learning in the Morris water maze by a neural network model of the hippocampal formation and nucleus accumbens. Hippocampus 5: 171-188.

Brun VH, Otnass MK, Molden S, Steffenach HA, Witter MP, Moser MB, Moser EI (2002) Place cells and place resognition maintained by direct entorhinal-hippocampal circuitry. Science 296: 2243-2246.

Bunsey M, Eichenbaum H (1995) Selective damage to the hippocampal region blocks long-term retention of a natural and nonspatial stimulus-stimulus association. Hippocampus 5: 546-556.

Burgess N, Recce M, O'Keefe J (1994) A model of hippocampal function. Neural Networks 7 : 1065-1081.

Burgess N, O'Keefe J (1996a) Neuronal computations underlying the firing of place cells and their role in navigation. Hippocampus 6: 749-762.

Burgess N, O'Keefe J (1996b) Cognitive graphs, resistive grids, and the hippocampal representation of space. J Gen Physiol 107: 659-662.

Burgess N, Donnett JG, Jeffery KJ, O'Keefe J (1997) Robotic and neuronal simulation of the hippocampus and rat navigation. Philos Trans R Soc Lond B Biol Sci 352: 1535-1543.

Burgess N, Donnett JG, O'Keefe J (1998) The representation of space and the hippocampus in rats, robots and humans. Z Naturforsch [C] 53: 504-509.

Burgess N, Jackson A, Hartley T, O'Keefe J (2000) Predictions derived from modelling the hippocampal role in navigation. Biol Cybern 83: 301-312.

Burgess N, Becker S, King JA, O'Keefe J (2001) Memory for events and their spatial context: models and experiments. Philos Trans R Soc Lond B Biol Sci 356: 1493-1503.

Burgess N, Becker S, Lever C, Cacucci F, O'Keefe J Spatial analysis of
cell firing in freely moving animals. Manuscript in preparation.

Burton S, Murphy D, Qureshi U, Sutton P, O'Keefe J (2000) Combined lesions of hippocampus and subiculum Do not produce deficits in a nonspatial social olfactory memory task. J Neurosci 20: 5468-5475.

Burwell RD, Amaral DG (1998a) Perirhinal and postrhinal cortices of the rat: interconnectivity and connections with the entorhinal cortex. J Comp Neurol 391: 293-321.

Burwell RD, Amaral DG (1998b) Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. J Comp Neurol 398: 179-205.

Burwell RD (2000) the parahippocampal region: corticocortical connectivity. Ann NY Acad Sci 911:25-42.

Buzsaki G, Leung LW, Vanderwolf CH (1983) Cellular bases of hippocampal EEG in the behaving rat. Brain Res 287: 139-171.

Buzsaki G, Czopf J, Kondakor I, Kellenyi L (1986) Laminar distribution of hippocampal rhythmic slow activity (RSA) in the behaving rat: current-source density analysis, effects of urethane and atropine. Brain Res 365: 125-137.

Buzsaki G, Gage FH, Czopf J, Bjorklund A (1987) Restoration of rhythmic slow activity (theta) in the subcortically denervated hippocampus by fetal CNS transplants. Brain Res 400: 334-347.

Buzsaki G (1989) Two-stage model of memory trace formation: a role for "noisy" brain states. Neuroscience 31: 551-570.

Caballero-Bleda M, Witter MP (1993) Regional and laminar organization of projections from the presubiculum and parasubiculum to the entorhinal cortex: an anterograde tracing study in the rat. J Comp Neurol 328: 115-129.

Caballero-Bleda M, Witter MP (1994) Projections from the presubiculum and the parasubiculum to morphologically characterized entorhinal-hippocampal projection neurons in the rat. Exp Brain Res 101: 93-108.

Chen LL, Lin LH, Green EJ, Barnes CA, McNaughton BL (1994a) Head-direction cells in the rat posterior cortex. I. Anatomical distribution and behavioral modulation. Exp Brain Res 101: 8-23.

Chen LL, Lin LH, Barnes CA, McNaughton BL (1994b) Head-direction cells in the rat posterior cortex. II. Contributions of visual and ideothetic information to the directional firing. Exp Brain Res 101: 24-34.

Cheng K (1986) A purely geometric module in the rat's spatial representation Cognition 23: 149-178.

Cho J, Sharp PE (2001) Head direction, place, and movement correlates for cells in the rat retrosplenial cortex. Behav Neurosci 115: 3-25.

Chrobak JJ, Buzsaki G (1994) Selective activation of deep layer (V-VI) retrohippocampal cortical neurons during hippocampal sharp waves in the behaving rat. J Neurosci 14: 6160-6170.

Chrobak JJ, Buzsaki G (1996) High-frequency oscillations in the output networks of the hippocampal-entorhinal axis of the freely behaving rat. J Neurosci 16: 3056-3066.

Chrobak JJ, Buzsaki G (1998) Gamma oscillations in the entorhinal cortex of the freely behaving rat. J Neurosci 18: 388-398.

Cohen NJ, Squire LR (1980) Preserved learning and retention of pattern-analyzing skill in amnesia: dissociation of knowing how and knowing that. Science 210: 207-210.

Colom LV, Bland BH (1987) State-dependent spike train dynamics of hippocampal formation neurons: evidence for theta-on and theta-off cells. Brain Res 422: 277-286.

Colom LV, Nassif-Caudarella S, Dickson CT, Smythe JW, Bland BH (1991) In vivo intrahippocampal microinfusion of carbachol and bicuculline induces theta-like oscillations in the septally deafferented hippocampus. Hippocampus 1: 381-390.

Cressant A, Muller RU, Poucet B (1997) Failure of centrally placed objects to control the firing fields of hippocampal place cells. J Neurosci 17: 2531-2542.

Cressant A, Muller RU, Poucet B (1999) Further study of the control of place cell firing by intra-apparatus objects. Hippocampus 9: 423-431.

Csicsvari J, Hirase H, Czurko A, Mamiya A, Buzsaki G (1999) Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving Rat. J Neurosci 19: 274-287.

Czurko A, Hirase H, Csicsvari J, Buzsaki G (1999) Sustained activation of hippocampal pyramidal cells by 'space clamping' in a running wheel. Eur J Neurosci 11: 344-352.

Dolorfo CL, Amaral DG (1998a) Entorhinal cortex of the rat: topographic organization of the cells of origin of the perforant path projection to the dentate gyrus. J Comp Neurol 398: 25-48.

Dolorfo CL, Amaral DG (1998b) Entorhinal cortex of the rat: organization of intrinsic connections. J Comp Neurol 398: 49-82.

Donovan MK, Wyss JM (1983) Evidence for some collateralization between cortical and diencephalic efferent axons of the rat subicular cortex. Brain Res 259: 181-192.

Draguhn A, Traub RD, Schmitz D, Jefferys JG (1998) Electrical coupling underlies high-frequency oscillations in the hippocampus in vitro. Nature 394: 189-192.

Dudchenko PA, Taube JS (1997) Correlation between head direction cell activity and spatial behavior on a radial arm maze. Behav Neurosci 111: 3-19.

Dudchenko PA, Goodridge JP, Taube JS (1997) The effects of disorientation on visual landmark control of head direction cell orientation. Exp Brain Res 115: 375-380.

Eichenbaum H, Wiener SI, Shapiro ML, Cohen NJ (1989) The organization of spatial coding in the hippocampus: a study of neural ensemble activity. J Neurosci 9: 2764-2775.

Eichenbaum H (1994) The hippocampal system and declarative memory in humans and animals: Experimental analysis and historical origins. In: Schacter DL, Tulving E (1994) Memory systems 1994. Cambridge, MIT press.

Fox SE, Ranck JB (1975) Localization and anatomical identification of theta and complex spike cells in dorsal hippocampal formation of rats. Exp Neurol 49: 299-313.

Fox SE, Wolfson S, Ranck JB (1986) Hippocampal theta rhythm and the firing of neurons in walking and urethane anesthetized rats. Exp Brain Res 62: 495-508.

Frank LM, Brown EN, Wilson M (2000) Trajectory encoding in the hippocampus and entorhinal cortex. Neuron 27: 169-178.

Frank LM, Brown EN, Wilson MA (2001) A comparison of the firing properties of putative excitatory and inhibitory neurons from CA1 and the entorhinal cortex. J Neurophysiol 86: 2029-2040.

Freund TF, Antal M (1988) GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. Nature 336: 170-173.

Frotscher M, Leranth C (1985) Cholinergic innervation of the rat hippocampus as revealed by choline acetyltransferase immunocytochemistry: a combined light and electron microscopic study. J Comp Neurol 239: 237-246.

Fujita Y, Sato T (1964) Intracellular records from hippocampal pyramidal cells in rabbit during theta activity. J Neurophysiol 27: 1011-1025.

Funahashi M, Stewart M (1997a) Presubicular and parasubicular cortical neurons of the rat: electrophysiological and morphological properties. Hippocampus 7: 117-129.

Funahashi M, Stewart M (1997b) Presubicular and parasubicular cortical neurons of the rat: functional separation of deep and superficial neurons in vitro. J Physiol 501: -403.

Funahashi M, Stewart M (1998) Properties of gamma-frequency oscillations initiated by propagating population bursts in retrohippocampal regions of rat brain slices. J Physiol 510: -208.

Galef B-GJ, Mason JR, Preti G, Bean NJ (1988) Carbon disulfide: a semiochemical mediating socially-induced diet choice in rats. Physiol Behav 42: 119-124.

Gaztelu JM, Buno W (1982) Septo-hippocampal relationships during EEG theta rhythm. Electroencephalogr Clin Neurophysiol 54: 375-387.

Goldberg M Studying the neurophysiology of behaviour: methods for recording single neurons in awake behaving monkeys. In: Current methods in neurobiology. Vol III. John Wiley and sons.

Golob EJ, Taube JS (1997) Head direction cells and episodic spatial information in rats without a hippocampus. Proc Natl Acad Sci U S A 94: 7645-7650.

Golob EJ, Wolk DA, Taube JS (1998) Recordings of postsubiculum head direction cells following lesions of the laterodorsal thalamic nucleus. Brain Res 780: 9-19.

Golob EJ, Taube JS (1999) Head direction cells in rats with hippocampal or overlying neocortical lesions: evidence for impaired angular path integration. J Neurosci 19: 7198-7211.

Golob EJ, Stackman RW, Wong AC, Taube JS (2001) On the behavioral significance of head direction cells: neural and behavioral dynamics during spatial memory tasks. Behav Neurosci 115: 285-304.

Goodridge JP, Taube JS (1997) Interaction between the postsubiculum and anterior thalamus in the generation of head direction cell activity. J Neurosci 17: 9315-9330.

Goodridge JP, Dudchenko PA, Worboys KA, Golob EJ, Taube JS (1998) Cue control and head direction cells. Behav Neurosci 112: 749-761.

Goodridge JP, Touretzky DS (2000) Modeling attractor deformation in the rodent head-direction system. J Neurophysiol 83: 3402-3410.

Gothard KM, Skaggs WE, Moore KM, McNaughton BL (1996) Binding of hippocampal CA1 neural activity to multiple reference frames in a landmark-based navigation task. J Neurosci 16: 823-835.

Gray JA (1982) The Neuropsychology of Anxiety: an Enquiry into the functions of the Septo-hippocampal system. (OUP, Oxford).

Gray JA (1982) The Neuropsychology of Anxiety: an Enquiry into the functions of the Septo-hippocampal system. 2nd Edition. (OUP, Oxford).

Green JD and Arduini AA (1954) Hippocampal electrical activity in arousal. J Neurophysiol 17: 533-557.

Groenewegen HJ, Vermeulen-Van-der-Zee E, te-Kortschot A, Witter MP (1987) Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of Phaseolus vulgaris leucoagglutinin. Neuroscience 23: 103-120.

Gulyas AI, Seress L, Toth K, Acsady L, Antal M, Freund TF (1991) Septal GABAergic neurons innervate inhibitory interneurons in the hippocampus of the macaque monkey. Neuroscience 41: 381-390.

Hampson RE, Simeral JD, Deadwyler SA (1999) Distribution of spatial and nonspatial information in dorsal hippocampus. Nature 402: 610-614.

Harley CW and Martin GM (1999) Open field Motor patterns and object marking, but not Object sniffing, are altered by ibotenate lesions of the hipocampus. Neurobiol of Learning and Memory. 72: 202-214

Hartley T, Burgess N, Lever C, Cacucci F, O'Keefe J (2000) Modeling place fields in terms of the cortical inputs to the hippocampus. Hippocampus 10: 369-379.

Hetherington PA, Shapiro ML (1997) Hippocampal place fields are altered by the removal of single visual cues in a distance-dependent manner. Behav Neurosci 111: 20-34.

Hill AJ (1978) First occurrence of hippocampal spatial firing in a new environment. Exp Neurol 62: 282-297.

Hill AJ, Best PJ (1981) Effects of deafness and blindness on the spatial correlates of hippocampal unit activity in the rat. Exp Neurol 74: 204-217.

Huerta PT, Lisman JE (1993) Heightened synaptic plasticity of hippocampal CA1 neurons during a cholinergically induced rhythmic state. Nature 364: 723-725.

Humphrey DR, Schmidt EM (1990)Extracellular single-unit recording methods, in AA Boulton, GB Baker, CH Vanderwolf (eds) Neurophysiological techniques: applications to neural systems. (The Humana Press Inc., Clifton, NJ)

Huxter JR, Burgess N, O'Keefe J (2002) Theta phase precession of hippocampal CA1 and CA3 place cell firing in the rat: effects of compressing the place field. European J Neurosci

Insausti R, Herrero MT, Witter MP (1997) Entorhinal cortex of the rat: cytoarchitectonic subdivisions and the origin and distribution of cortical efferents. Hippocampus 7: 146-183.

Jarrard LE (1993) On the role of the hippocampus in learning and memory in the rat. Behav Neural Biol 60: 9-26.

Jeffery KJ, Donnett JG, Burgess N, O'Keefe JM (1997) Directional control of hippocampal place fields. Exp Brain Res 117: 131-142.

Jeffery KJ, O'Keefe JM (1999) Learned interaction of visual and idiothetic cues in the control of place field orientation. Exp Brain Res 127: 151-161.

Jeffery KJ, Gilbert A, Burton S, Strudwick A (2003) Preserved performance in a hippocampal dependent spatial task despite complete place cell remapping. Hippocampus. In press.

Jensen O, Lisman (2000) Position reconstruction from an ensemble of hippocampal place cells: contribution of theta phase coding. J Neurophysiol 83: 2602-2609.

Jones RS (1993) Entorhinal-hippocampal connections: a speculative view of their function. Trends Neurosci 16: 58-64.

Jongen-Relo AL, Pitkanen A, Amaral DG (1999) Distribution of GABAergic cells and fibers in the hippocampal formation of the macaque monkey: an immunohistochemical and in situ hybridization study. J Comp Neurol 408: 237-271.

Jung MW, McNaughton BL (1993) Spatial selectivity of unit activity in the hippocampal granular layer. Hippocampus 3: 165-182.

Jung MW, Wiener SI, McNaughton BL (1994) Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. J Neurosci 14: 7347-7356.

Kesner RP, Giles R (1998) Neural circuit analysis of spatial working memory: role of pre- and parasubiculum, medial and lateral entorhinal cortex. Hippocampus 8: 416-423.

King C, Recce M, O'Keefe J (1998) The rhythmicity of cells of the medial septum/diagonal band of Broca in the awake freely moving rat: relationships with behaviour and hippocampal theta. Eur J Neurosci 10: 464-477.

Knierim JJ, Kudrimoti HS, McNaughton BL (1995) Place cells, head direction cells, and the learning of landmark stability. J Neurosci 15: 1648-1659.

Knierim JJ, Kudrimoti HS, McNaughton BL (1998) Interactions between idiothetic cues and external landmarks in the control of place cells and head direction cells. J Neurophysiol 80: 425-446.

Kocsis B, Bragin A, Buzsaki G (1999) Interdependence of multiple theta generators in the hippocampus: a partial coherence analysis. J Neurosci 19: 6200-6212.

Kohler C (1985) Intrinsic projections of the retrohippocampal region in the rat brain. I. The subicular complex. J Comp Neurol 236: 504-522.

Kohler C (1986) Intrinsic connections of the retrohippocampal region in the rat brain. II. The medial entorhinal area. J Comp Neurol 246: 149-169.

Kohler C (1988) Intrinsic connections of the retrohippocampal region in the rat brain: III. The lateral entorhinal area. J Comp Neurol 271: 208-228.

Krettek JE, Price JL (1977) Projections from the amygdaloid complex to the cerebral cortex and thalamus in the rat and cat. J Comp Neurol 172: 687-722.

Kubie JL, Muller RU, Bostock E (1990) Spatial firing properties of hippocampal theta cells. J Neurosci 1990 10: 1110-1123.

Lacaille JC, Williams S (1990) Membrane properties of interneurons in stratum oriens-alveus of the CA1 region of rat hippocampus in vitro. Neuroscience 36: 349-359.

Lee MG, Chrobak JJ, Sik A, Wiley RG, Buzsaki G (1994) Hippocampal theta activity following selective lesion of the septal cholinergic system. Neuroscience 62: 1033-1047.

Lenck-Santini PP, Save E, Poucet B (2001) Evidence for a relationship between place-cell spatial firing and spatial memory performance. Hippocampus 11: 377-390.

Leutgeb S, Ragozzino KE, Mizumori SJ (2000) Convergence of head direction and place information in the CA1 region of hippocampus. Neuroscience 100: 11-19.

Lever C, Wills T, Cacucci F, Burgess N, O'Keefe J (2002a) Long-term plasticity in hippocampal place-cell representation of environmental geometry. Nature 416: 90-94.

Lever C, Burgess N, Cacucci F, Hartley T, O'Keefe J (2002b) What can the hippocampal representation of environmental geometry tell us about Hebbian learning? Biol Cybern 87: 356-372.

Lever C, Cacucci F, Wills T, Burton S, McClellan A, Burgess N, O'Keefe (2003) Spatial coding in the hippocampal formation: input, information type, plasticity, and behaviour in KJ Jeffery (ed) The Neurobiological basis of spatial behaviour (OUP, Oxford: in press).

Li XG, Somogyi P, Ylinen A, Buzsaki G (1994) The hippocampal CA3 network: an in vivo intracellular labeling study. J Comp Neurol 339: 181-208.

Liu P, Jarrard LE, Bilkey DK (2001) Excitotoxic lesions of the pre- and parasubiculum disrupt object recognition and spatial memory processes. Behav Neurosci 115: 112-124.

MacVicar BA, Tse FW (1989) Local neuronal circuitry underlying cholinergic rhythmical slow activity in CA3 area of rat hippocampal slices. J Physiol 417197-212: -212.

Maguire EA, Burgess N, Donnett JG, Frackowiak RS, Frith CD, O'Keefe J (1998) Knowing where and getting there: a human navigation network. Science 280: 921-924.

Maguire EA (2001) Neuroimaging studies of autobiographical event memory. Philos Trans R Soc Lond B Biol Sci 356: 1441-1451.

Markus EJ, Barnes CA, McNaughton BL, Gladden VL, Skaggs WE (1994) Spatial information content and reliability of hippocampal CA1 neurons: effects of visual input. Hippocampus 4: 410-421.

Markus EJ, Qin YL, Leonard B, Skaggs WE, McNaughton BL, Barnes CA (1995) Interactions between location and task affect the spatial and directional firing of hippocampal neurons. J Neurosci 15: 7079-7094.

McNaughton BL, Barnes CA (1977) Physiological identification and analysis of dentate granule cell responses to stimulation of the medial and lateral perforant pathways in the rat. J Comp Neurol 175: 439-454.

McNaughton BL, Barnes CA, O'Keefe J (1983) The contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats. Exp Brain Res 52: 41-49.

McNaughton BL, Barnes CA, Meltzer J, Sutherland RJ (1989) Hippocampal granule cells are necessary for normal spatial learning but not for spatially-selective pyramidal cell discharge. Exp Brain Res 76: 485-496.

McNaughton BL, Barnes CA, Gerrard JL, Gothard K, Jung MW, Knierim JJ, Kudrimoti H, Qin Y, Skaggs WE, Suster M, Weaver KL (1996) Deciphering the hippocampal polyglot: the hippocampus as a path integration system. J Exp Biol 199: 173-185.

McNaughton N and Gray JA (2000) Anxiolytic action on the behavioural inhibition system implies multiple types of arousal contribute to anxiety. Journal of Affective Disorders 61: 161-176.

Mehta MR, Barnes CA, McNaughton BL (1997) Experience-dependent, asymmetric expansion of hippocampal place fields. Proc Natl Acad Sci U S A 94: 8918-8921.

Mehta MR, Quirk MC, Wilson MA (2000) Experience-dependent asymmetric shape of hippocampal receptive fields. Neuron 25: 707-715.

Meibach RC, Siegel A (1975) The origin of fornix fibers which project to the mammillary bodies in the rat: a horseradish peroxidase study. Brain Res 88: 508-512.

Mizumori SJ, Ward KE, Lavoie AM (1992) Medial septal modulation of entorhinal single unit activity in anesthetized and freely moving rats. Brain Res 570: 188-197.

Mizumori SJ, Williams JD (1993) Directionally selective mnemonic properties of neurons in the lateral dorsal nucleus of the thalamus of rats. J Neurosci 13: 4015-4028.

Mizumori SJ, Miya DY, Ward KE (1994) Reversible inactivation of the lateral dorsal thalamus disrupts hippocampal place representation and impairs spatial learning. Brain Res 644: 168-174.

Mizumori SJ, Ragozzino KE, Cooper BG, Leutgeb S (1999) Hippocampal representational organization and spatial context. Hippocampus 9: 444-451.

Morris RG, Garrud P, Rawlins JN, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. Nature 297: 681-683.

Morris RG, Schenk F, Tweedie F, Jarrard LE (1990) Ibotenate lesions of hippocampus and/or subiculum: dissociating components of allocentric spatial learning. Eur J Neurosci 2: 1016-1028.

Morris RG, Frey U (1997) Hippocampal synaptic plasticity: role in spatial learning or the automatic recording of attended experience? Philos.Trans.R.Soc.Lond.B.Biol.Sci. 352:1489-1503.

Moser E, Moser MB, Andersen P (1993) Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. J Neurosci 13: 3916-3925.

Mulders WH, West MJ, Slomianka L (1997) Neuron numbers in the presubiculum, parasubiculum, and entorhinal area of the rat. J Comp Neurol 385: 83-94.

Muller RU, Kubie JL, Ranck J-BJ (1987) Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. J Neurosci 7: 1935-1950.

Muller RU, Kubie JL (1987) The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. J Neurosci 7: 1951-1968.

Muller RU, Kubie JL (1989) The firing of hippocampal place cells predicts the future position of freely moving rats. J Neurosci 9: 4101-4110.

Muller RU, Kubie JL, Saypoff R (1991) The hippocampus as a cognitive graph (abridged version). Hippocampus 1: 243-246.

Muller RU, Bostock E, Taube JS, Kubie JL (1994) On the directional firing properties of hippocampal place cells. J Neurosci 14: 7235-7251.

Muller RU, Stead M, Pach J (1996) The hippocampus as a cognitive graph. J Gen Physiol 107: 663-694.

Muller RU, Stead M (1996) Hippocampal place cells connected by Hebbian synapses can solve spatial problems. Hippocampus 6: 709-719.

Mumby DG, Wood ER, Duva CA, Kornecook TJ, Pinel JP, Phillips AG (1996) Ischemia-induced object-recognition deficits in rats are attenuated by hippocampal ablation before or soon after ischemia. Behav Neurosci 110: 266-281.

Naber PA, Caballero-Bleda M, Jorritsma-Byham B, Witter MP (1997) Parallel input to the hippocampal memory system through peri- and postrhinal cortices. Neuroreport 28: 2617-2621.

Naber PA, Witter MP (1998) Subicular efferents are organized mostly as parallel projections: a double-labeling, retrograde-tracing study in the rat. J Comp Neurol 393: 284-297.

Naber PA, Witter MP, Lopez-da-Silva FH (1999) Perirhinal cortex input to the hippocampus in the rat: evidence for parallel pathways, both direct and indirect. A combined physiological and anatomical study. Eur J Neurosci 11: 4119-4133.

Naber PA, Witter MP, Lopes-da-Silva FH (2001) Evidence for a direct projection from the postrhinal cortex to the subiculum in the rat. Hippocampus 11: 105-117.

Nadel L (1994) Multiple memory systems: What and why, an update. In: Schcter DL, Tulving E (1994) Memory systems 1994. Cambridge, MIT Press.

Nadel L, Moscovitch M (1997) Memory consolidation, retrograde amnesia and the hippocampal complex. Curr Opin Neurobiol 7: 217-227.

Nakazawa K ,Quirk MC, Chitwood RA, Watanabe M, Yeckel MF, Sun LD, Kato A, Carr CA, Johnston D, Wilson MA, Tonegawa S (2002) Requirement for hippocampal CA3 NMDA receptors in associative memory recall. Science 297: 211-218.

O'Keefe J, Dostrovsky J (1971) The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Res 34: 171-175.

O'Keefe J (1976) Place units in the hippocampus of the freely moving rat. Exp Neurol 51: 78-109.

O'Keefe J, Black AH (1977) Single unit and lesion experiments on the sensory inputs to the hippocampal cognitive map. Ciba Found Symp 179-198.

O'Keefe J, Conway DH (1978) Hippocampal place units in the freely moving rat: why they fire where they fire. Exp Brain Res 31: 573-590.

O'Keefe J, Nadel L (1978) The hippocampus as a cognitive map. Oxford: Clarendon Press.

O'Keefe J (1983) Two spatial systems in the rat brain--implications for the neural basis of learning and memory. Prog Brain Res 58: 453-464.

O'Keefe J, Speakman A (1987) Single unit activity in the rat hippocampus during a spatial memory task. Exp Brain Res 68: 1-27.

O'Keefe J (1990) A computational theory of the hippocampal cognitive map. Prog Brain Res 83: 301-312.

O'Keefe J (1991) An allocentric spatial model for the hippocampal cognitive map. Hippocampus 1: 230-235.

O'Keefe J (1993) Hippocampus, theta, and spatial memory. Curr Opin Neurobiol 3: 917-924.

O'Keefe J, Recce ML (1993) Phase relationship between hippocampal place units and the EEG theta rhythm. Hippocampus 3: 317-330.

O'Keefe J, Burgess N (1996) Geometric determinants of the place fields of hippocampal neurons. Nature 381: 425-428.

O'Keefe J, Burgess N, Donnett JG, Jeffery KJ, Maguire EA (1998) Place cells, navigational accuracy, and the human hippocampus. Philos Trans R Soc Lond B Biol Sci 353: 1333-1340.

O'Keefe J (1999) Do hippocampal pyramidal cells signal non-spatial as well as spatial information? Hippocampus 9: 352-364.

Olton DS, Branch M, Best, PJ (1978) Spatial correlates of hippocampal unit activity. Exp Neurol 58: 387-409.

Pavlides C, Greenstein YJ, Grudman M, Winson J (1988) Long-term potentiation in the dentate gyrus is induced preferentially on the positive phase of theta-rhythm. Brain Res 439: 383-387.

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. New York: Academic Press.

Poucet B, Thinus-Blanc C, Muller RU (1994) Place cells in the ventral hippocampus of rats. Neuroreport 27: 2045-2048.

Quirk GJ, Muller RU, Kubie JL (1990) The firing of hippocampal place cells in the dark depends on the rat's recent experience. J Neurosci 10: 2008-2017.

Quirk GJ, Muller RU, Kubie JL, Ranck J-BJ (1992) The positional firing properties of medial entorhinal neurons: description and comparison with hippocampal place cells. J Neurosci 12: 1945-1963.

Ranck-JB J (1973) Studies on single neurons in dorsal hippocampal formation an septum in unrestrained rats. I . Behavioral correlates and firing repertoires. Exp Neurol 41: 461-531.

Ranck-JB J (1984) Head-direction cells in the deep layers of dorsal presubiculum in freely moving rats Soc Neurosci Abstr 10: 599.

Redish AD, Elga AN, Touretzky DS (1996) A coupled attractor model of the rodent head direction system. Network: Computation in Neural Network Systems 7:671-685.

Redish AD (1999) Beyond the cognitive map. From place cells to episodic memory. Cambridge: MIT Press.

Riedel G, Micheau J, Lam AGM, Roloff EvL, Martin SJ, Bridge H, de Hoz L, Poeschel B, McCulloch J, Morris RGM (1999) Reversible neural inactivation reveals hippocampal participation in several memory processes. Nature Neurosci 2: 898-905.

Rose G, Diamond D, Lynch GS (1983) Dentate granule cells in the rat hippocampal formation have the behavioral characteristics of theta neurons. Brain Res 266: 29-37.

Rose GM, Dunwiddie TV (1986) Induction of hippocampal long-term potentiation using physiologically patterned stimulation. Neurosci Lett 69: 244-248.

Rosenbaum RS, Priselac S, Kohler S, Black SE, Gao F, Nadel L, Moscovitch M (2000) Remote spatial memory in an amnesic person with extensive bilateral hippocampal lesions. Nat Neurosci 3: 1044-1048.

Ruth RE, Collier TJ, Routtenberg A (1982) Topography between the entorhinal cortex and the dentate septotemporal axis in rats: I. Medial and intermediate entorhinal projecting cells. J Comp Neurol 209: 69-78.

Ruth RE, Collier TJ, Routtenberg A (1988) Topographical relationship between the entorhinal cortex and the septotemporal axis of the dentate gyrus in rats: II. Cells projecting from lateral entorhinal subdivisions. J Comp Neurol 270: 506-516.

Ryan L, Nadel L, Keil L, Putnam K, Schnyer D, Trouard T, Moscovitch M (2001) Hippocampal complex and retrieval of recent and very remote autobiographical memories: evidence from functional magnetic resonance imaging in neurologically intact people. Hippocampus 11: 707-714.

Salmon DP, Zola=Morgan S, Squire LR (1985) Rretrograde amnesia following combined hippocampus-amygdala lesions in monkeys. Psychobiology 15: 37-47.

Samsonovich A, McNaughton BL (1997) Path integration and cognitive mapping in a continuous attractor neural network model. J Neurosci 17:5900-5920.

Sarter M, Markowitsch HJ (1985) Convergence of intra- and interhemispheric cortical afferents: lack of collateralization and evidence for a subrhinal cell group projecting heterotopically. J Comp Neurol 236: 283-296.

Save E, Cressant A, Thinus-Blanc C, Poucet B (1998) Spatial firing of hippocampal place cells in blind rats. J Neurosci 18: 1818-1826.

Schacter DL (1987) Implicit memory: history and current status. J of Exp Psychol: Learnin, Memory, Cognition 13: 501-518.

Schacter DL (1994) Priming and multiple memory systems: perceptual mechanisms of implicit memory. IN: Schacter DL, Tulving E (1994) Memory systems 1994. Cambridge, MIT Press.

Scharfman HE, Witter MP, Schwarcz R (2000) The parahippocampal region. Implications for neurological and psychiatric diseases. Introduction. Ann N Y Acad Sci 911ix-xiii: -xiii.

Schmitz D, Schuchmann S, Fisahn A, Draguhn A, Buhl EH, Petrasch-Parwez E, Dermietzel R, Heinemann U, Traub RD (2001) Axo-axonal coupling. a novel mechanism for ultrafast neuronal communication. Neuron 31: 831-840.

Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry; 20:11-21.

Sharp PE, Kubie JL, Muller RU (1990) Firing properties of hippocampal neurons in a visually symmetrical environment: contributions of multiple sensory cues and mnemonic processes. J Neurosci 10: 3093-3105.

Sharp PE, Green C (1994) Spatial correlates of firing patterns of single cells in the subiculum of the freely moving rat. J Neurosci 14: 2339-2356.

Sharp PE, Blair HT, Brown M (1996) Neural network modeling of the hippocampal formation spatial signals and their possible role in navigation: a modular approach. Hippocampus 6: 720-734.

Sharp PE (1996) Multiple spatial/behavioral correlates for cells in the rat postsubiculum: multiple regression analysis and comparison to other hippocampal areas. Cereb Cortex 6: 238-259.

Sharp PE (1997) Subicular cells generate similar spatial firing patterns in two geometrically and visually distinctive environments: comparison with hippocampal place cells. Behav Brain Res 85: 71-92.

Sharp PE (1999a) Comparison of the timing of hippocampal and subicular spatial signals: implications for path integration. Hippocampus 9: 158-172.

Sharp PE (1999b) Subicular place cells expand or contract their spatial firing pattern to fit the size of the environment in an open field but not in the presence of barriers: comparison with hippocampal place cells. Behav Neurosci 113: 643-662.

Sharp PE (1999c) Complimentary roles for hippocampal versus subicular/entorhinal place cells in coding place, context, and events. Hippocampus 9: 432-443.

Sharp PE, Blair HT, Cho J (2001a) The anatomical and computational basis of the rat head-direction cell signal. Trends Neurosci 24: 289-294.

Sharp PE, Tinkelman A, Cho J (2001b) Angular velocity and head direction signals recorded from the dorsal tegmental nucleus of gudden in the rat: implications for path integration in the head direction cell circuit. Behav Neurosci 115: 571-588.

Shibata H (1993) Direct projections from the anterior thalamic nuclei to the retrohippocampal region in the rat. J Comp Neurol 337: 431-445.

Shute CC, Lewis PR (1966) Cholinergic and monoaminergic pathways in the hypothalamus. Br Med Bull 22:221-226.

Skaggs WE, McNaughton BL (1992) Computational approaches to hippocampal function Curr Opin Neurobiol 2: 209-211.

Skaggs WE, McNaughton BL, Gothard KM, Markus EJ (1993) An information-theoretic approach to deciphering the hippocampal code. Neural Information Processing Systems 5: 1030-1037.

Skaggs WE, Knierim JJ, Kudrimoti HS, McNaughton BL (1995) A model of the neural basis of the rat's sense of direction. Adv Neural Inf Process Syst 7: 173-180.

Skaggs WE, McNaughton BL, Wilson MA, Barnes CA (1996) Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. Hippocampus 6: 149-172.

Smythe JW, Cristie BR, Colom LV, Lawson VH, Bland BH (1991) Hippocampal theta field activity and theta-on/theta-off cell discharges are controlled by an ascending hypothalamo-septal pathway. J Neurosci 11: 2241-2248.

Sokolov EN (1963) Perception and the conditioned reflex. London, Pergamon

Squire LR (1987) Memory and Brain. New York, Oxford University Press.

Squire LR (1994) Declarative and nondeclarative memory: multiple brain systems supporting learning and memory. IN: Schacter DL, Tulving E (1994) Memory systems 1994. Cambridge, MIT Press.

Stackman RW, Taube JS (1997) Firing properties of head direction cells in the rat anterior thalamic nucleus: dependence on vestibular input. J Neurosci 17: 4349-4358.

Stackman RW, Taube JS (1998) Firing properties of rat lateral mammillary single units: head direction, head pitch, and angular head velocity. J Neurosci 18: 9020-9037.

Steward O, Scoville SA (1976) Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat. J Comp Neurol 169: 347-370.

Stewart M, Fox SE (1989) Firing relations of medial septal neurons to the hippocampal theta rhythm in urethane anesthetized rats. Exp Brain Res 77: 507-516.

Stewart M, Fox SE (1990) Do septal neurons pace the hippocampal theta rhythm? Trends Neurosci 13: 163-168.

Swanson LW, Cowan WM (1977) An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. J Comp Neurol 172: 49-84.

Swanson LW, Wyss JM, Cowan WM (1978) An autoradiographic study of the organization of intrahippocampal association pathways in the rat. J Comp Neurol 181: 681-716.

Swanson LW, Kohler C (1986) Anatomical evidence for direct projections from the entorhinal area to the entire cortical mantle in the rat. J Neurosci 6: 3010-3023.

Tamamaki N, Watanabe K, Nojyo Y (1984) A whole image of the hippocampal pyramidal neuron revealed by intracellular pressure-injection of horseradish peroxidase. Brain Res 307: 336-340.

Tamamaki N, Abe K, Nojyo Y (1987) Columnar organization in the subiculum formed by axon branches originating from single CA1 pyramidal neurons in the rat hippocampus. Brain Res 412: 156-160.

Tamamaki N, Abe K, Nojyo Y (1988) Three-dimensional analysis of the whole axonal arbors originating from single CA2 pyramidal neurons in the rat hippocampus with the aid of a computer graphic technique. Brain Res 452: 255-272.

Tamamaki N, Nojyo Y (1990) Disposition of the slab-like modules formed by axon branches originating from single CA1 pyramidal neurons in the rat hippocampus. J Comp Neurol 291: 509-519.

Tamamaki N, Nojyo Y (1995) Preservation of topography in the connections between the subiculum, field CA1, and the entorhinal cortex in rats. J Comp Neurol 353: 379-390.

Taube JS, Muller RU, Ranck J-BJ (1990a) Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis. J Neurosci 10: 420-435.

Taube JS, Muller RU, Ranck J-BJ (1990b) Head-direction cells recorded from the postsubiculum in freely moving rats. II. Effects of environmental manipulations. J Neurosci 10: 436-447.

Taube JS, Kesslak JP, Cotman CW (1992) Lesions of the rat postsubiculum impair performance on spatial tasks. Behav Neural Biol 57: 131-143.

Taube JS (1995a) Head direction cells recorded in the anterior thalamic nuclei of freely moving rats. J Neurosci 15: 70-86.

Taube JS, Burton HL (1995b) Head direction cell activity monitored in a novel environment and during a cue conflict situation. J Neurophysiol 74: 1953-1971.

Taube JS (1995c) Place cells recorded in the parasubiculum of freely moving rats. Hippocampus 5: 569-583.

Taube JS, Stackman RW, Dudchenko PA (1996) Head-direction cell activity monitored following passive transport into a novel environment. Soc. Neurosci Abstr 22: 1873.

Taube JS, Muller RU (1998) Comparisons of head direction cell activity in the postsubiculum and anterior thalamus of freely moving rats. Hippocampus 8: 87-108.

Taube JS (1998) Head direction cells and the neurophysiological basis for a sense of direction. Prog Neurobiol 55: 225-256.

Teng E, Squire LR (1999) Memory for places learned long ago is intact after hippocampal damage. Nature 400: 675-677.

Teyler TJ and DiScenna P (1986) The hippocampal memory indexing theory Behavioral Neurosci 100: 147-154.

Tolman EC (1948) Cognitive maps in rats and men. Psychol Rev 40: 40-60.

Touretzky DS, Redish AD (1996) Theory of rodent navigation based on interacting representations of space. Hippocampus 6: 247-270.

Traub RD, Whittington MA, Colling SB, Buzsaki G, Jefferys JG (1996) Analysis of gamma rhythms in the rat hippocampus in vitro and in vivo. J Physiol 493: -84.

Tulving E (1984) Precis of elements of episodic memory. Behavioral Brain Sciences 7: 223-268.

Van Groen T, Wyss JM (1990a) The connections of presubiculum and parasubiculum in the rat. Brain Res 518: 227-243.

Van Groen T, Wyss JM (1990b) The postsubicular cortex in the rat: characterization of the fourth region of the subicular cortex and its connections. Brain Res 529: 165-177.

Van Groen T, Wyss JM (1990c) Extrinsic projections from area CA1 of the rat hippocampus: olfactory, cortical, subcortical and bilateral hippocampal formation projections. J Comp Neurol 302: 515-528.

Van Groen T, Wyss JM (1995) Projections from the anterodorsal and anteroventral nucleus of the thalamus to the limbic cortex of the rat. J Comp Neurol 358: 584-604.

van Haeften T, Wouterlood FG, Jorritsma-Byham B, Witter MP (1997) GABAergic presubicular projections to the medial entorhinal cortex of the rat. J Neurosci 17: 862-874.

van Haeften T, Wouterlood FG, Witter MP (2000) Presubicular input to the dendrites of layer V entorhinal neurons in the rat. Ann N Y Acad Sci 911: 471-3.

Van Hoesen GW (1982) the parahippocampal gyrus: new observations regarding its cortical connections in the monkey. TINS 5: 345-350.

Vanderwolf CH (1969) Hippocampal electrical activity and voluntary movement in the rat. Electroencephalogr Clin Neurophysiol 26: 407-418.

Vann SD, Brown MW, Aggleton JP (2000) Fos expression in the rostral thalamic nuclei and associated cortical regions in response to different spatial memory tests. Neuroscience 101: 983-991.

Vargha-Khadem F, Gadian DG, Watkins KE, Connelly A, Van Paesschen W, Mishkin M (1997) Differential effects of early hippocampal pathology on episodic and semantic memory. Science 277: 376-380.

Vertes RP, Albo Z, Viana Di Prisco G (2001) Theta-rhythmically firing neurons in the anterior thalamus: implications for mnemonic functions of Papez's circuit. Neuroscience 104 (3): 619-25.

Vertes RP, Kocsis B (1997) Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus. Neuroscience 81: 893-926.

Vinogradova OS (1995) Expression, control, and probable functional significance of the neuronal theta-rhythm. Prog Neurobiol 45: 523-583.

Vinogradova OS (2001) Hippocampus as comparator: role of the two input and two output systems of the hippocampus in selection and registration of information. Hippocampus 11:578-98

Vogt BA, Miller MW (1983) Cortical connections between rat cingulate cortex and visual, motor, and postsubicular cortices. J Comp Neurol 216: 192-210.

Whishaw IQ, Jarrard LE (1996) Evidence for extrahippocampal involvement in place learning and hippocampal involvement in path integration. Hippocampus 6: 513-524.

Wiener SI (1993) Spatial and behavioral correlates of striatal neurons in rats performing a self-initiated navigation task. J Neurosci 13: 3802-3817.

Wilson MA, McNaughton BL (1993) Dynamics of the hippocampal ensemble code for space. Science 261: 1055-1058.

Witter MP, Holtrop R, van de Loosdrecht AA (1988) Direct projections from the periallocortical subicular complex to the fascia dentate in the rat: an anatomical tracing study using Phaseolus vulgaris leucoagglutinin. Neurosci Res Commun 2: 61-68.

Witter MP, Groenewegen HJ, Lopes-da-Silva FH, Lohman AH (1989) Functional organization of the extrinsic and intrinsic circuitry of the parahippocampal region. Prog Neurobiol 33: 161-253.

Witter MP, Groenewegen HJ (1990) The subiculum: cytoarchitectonically a simple structure, but hodologically complex. Prog Brain Res 8347-58: -58.

Witter MP, Amaral DG (1991) Entorhinal cortex of the monkey: V. Projections to the dentate gyrus, hippocampus, and subicular complex. J Comp Neurol 307: 437-459.

Witter MP, Naber PA, van Haeften T, Machielsen WC, Rombouts SA, Barkhof F, Scheltens P, Lopes-da-Silva FH (2000) Cortico-hippocampal communication by way of parallel parahippocampal-subicular pathways. Hippocampus 10: 398-410.

Witter MP, Wouterlood FG, Naber PA, van Haeften T (2000) Anatomical organization of the parahippocampal-hippocampal network. Ann N Y Acad Sci 9111-24: -24.

Wood ER, Dudchenko PA, Eichenbaum H (1999) The global record of memory in hippocampal neuronal activity. Nature 397: 613-616.

Wouterlood FG, Saldana E, Witter MP (1990) Projection from the nucleus reuniens thalami to the hippocampal region: light and electron microscopic tracing study in the rat with the anterograde tracer Phaseolus vulgaris-leucoagglutinin. J Comp Neurol 296: 179-203.

Wyss JM, Van Groen T (1992) Connections between the retrosplenial cortex and the hippocampal formation in the rat: a review. Hippocampus 2: 1-11.

Ylinen A, Bragin A, Nadasdy Z, Jando G, Szabo I, Sik A, Buzsaki G (1995) Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. J Neurosci 15: 30-46.

Zhang K (1996) Representation of spatial orientation by the intrinsic dynamics of the head-direction cell ensemble: a theory. J Neurosci 16: 2112-2126.

Zola-Morgan S, Squire LR, Amaral DG (1986) Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. J Neurosci 6: 2950-2967.

Zola-Morgan S, Squire LR (1990) the primate hippocampal formation: evidence for a time-limited role in memory storage. Science 250: 288-290.

Zola-Morgan S, Squire LR, Rempel NL, Clower RP, Amaral DG (1992) Enduring memory impairment in monkeys after ischemic damage to the hippocampus. J Neurosci 12: 2582-2596.

Zola SM, Squire LR, Teng E, Stefanacci L, Buffalo EA, Clark RE (2000) Impaired recognition memory in monkeys after damage limited to the hippocampal region. J Neurosci 20: 451-463.

APPENDIX

Cell selection criteria

Cells were selected on the basis of their locational and directional correlates, and rhythmicity of their firing. For a cell to be classified as topodirectional (TD), and be included in this study it had to meet the following criteria:

a) peak rate in the locational field should exceed 1 Hz;
b) peak rate in the directional field should exceed 1 Hz;
c) it should show a clear theta modulation in the autocorrelogram.

During the sessions when at least one TD cell was present, 55 other cells were recorded that did not meet the above criteria. Of these, roughly 50% showed theta modulation (ranging from mild, 7 cells, to strong, 9 cells), the other 50% showed no rhythmicity in their firing.

A TD cell was judged to be well isolated when a clear 2 ms refractory period could be seen in its autocorrelogram.

TD cells tended to occur in clusters, ie in most cases more than one TD cell was recorded simultaneously (also found to be true for HD cells).

Roughly 20% of the putative non-TD cells recorded (11 units) were subjectively judged to be two or more TD cells that could not be isolated from each other. This conclusion was formed on the basis of these observations:

1) these units were all theta modulated;
2) they all presented discrete but multiple locational and directional fields;
3) their autocorrelations did not show a clear refractory period.

In these cases, spikes could not be satisfactorily sorted on the basis of either amplitude or voltage on the cluster space (see chapter VI, cluster cutting, p.112). In addition, most of these units (10 out of 11), were recorded from animal A, in which stereotrodes were used (as opposed to tetrodes).

ACKNOWLEDGEMENTS

I would like to thank John O'Keefe for being a great supervisor.
Neil Burgess for developing all the analytical tools used in this thesis.
All members of the O'Keefe Lab, past and present:
Colin Lever for showing me my first place cell, and for being always, always very helpful and inspiring (and extremely enthusiastic),
John Huxter for endless discussions and for sharing with me the pain of writing a PhD thesis,
Steve Burton for, among other things, cutting brains and complaining about it.
Tom Wills for his help,
Kate Jeffery for her suggestions,
Charles King for his suggestions about the theta section of the thesis (and for giving me some of his data).
Jim Donnett, Clive Parker, and John Ryan for invaluable and reliable technical assistance.
David Attwell for constantly reminding me that a life without a PhD is not worth living.

Thanks to my family and to my granddad, nonno Strato, to whom this thesis is dedicated.

The author was supported by a grant from the Wellcome Trust whilst working on this thesis.
Professor O'Keefe's laboratory, in which this research was conducted, was funded by a grant from the MRC.