Hippocampal neuronal mechanisms of coordination of two concurrently relevant spatial representations

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Contents

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Abstract

In natural conditions several different representations are often simultaneously relevant for an animal. In everyday situations like foraging for food an animal has to concentrate on its prey, watch for potential predators, be aware of its surroundings and ways to escape to safety. All these cognitive tasks have to be performed at essentially the same time, and the animal has to be able to shift priority from one task to another based on subtle changes of its environment. The main aim of this thesis is to study the neural mechanisms underlying the coordination of different simultaneously relevant neural representations. Specifically, coordination of two hippocampal spatial representations was studied.

The potential mechanisms of coordination of different representation are to a large extent determined by the nature of the representations. Different theories of information coding in nervous system set different limits on the way multiple simultaneously relevant representations can be organized. According to the local (dedicated) coding hypothesis different representations can be active together because they are coded by independent cells (Barlow, 1972). According to the distributed coding hypothesis different representations are coded by unique patterns of activity within the same large population of neurons (Hebb, 1949). This hypothesis predicts that only one representation can be active at a particular time. In chapters 1.1. – 1.5. different theories of information coding will be presented, discussed and evaluated against available experimental data.

The best experimental situation to study the coordination of multiple representations is a task in which at least two different representations are simultaneously relevant. We choose two-frame room+arena+ place avoidance task in which a rat is exposed to two different sets of landmarks that are continuously dissociated (Fenton et al., 1998). This is achieved by placing a rat on a slowly rotating arena (1 rpm), so that its position can be defined in two frames of reference – in the reference frame of the room, and in the reference frame of the rotating arena. To ensure that both reference frames are processed by the animal, the rat is reinforced to walk on the arena and to avoid two "shock zones", one defined relative to the room, the other relative to the arena. The room+arena+ place avoidance task is introduced in chapter 2.1.

The hippocampus plays a role in spatial memory and information processing (Morris et al., 1982; O'Keefe and Nadel, 1978) and hippocampal complex-spike cells, putative pyramidal cells (Ranck, 1973) are well known for their spatial responses (O'Keefe and Dostrovsky, 1971; Muller et al., 1987). In our experiments, ensembles of hippocampal CA1 cells were recorded and analyzed to determine the neuronal mechanisms that underlie coordination of two distinct spatial representations. Hippocampal activity was analyzed on different timescales in an effort to discover evidence for temporal coordination of different representations. The techniques of hippocampal ensemble recording and analysis are described in chapters 2.2.–2.5.

The results of these experiments lead to five conclusions:

1. Rats process information about their position in both frames of reference. (Chapter 3.1.)
2. Both spatial reference frames are represented in the activity of the hippocampal neurons. (Chapter 3.2.)
3. Simultaneously recorded neurons behave cohesively, they tend to code for the same frame of reference. (Chapter 3.3.)
4. The firing of hippocampal place cells is coordinated on the time-scale of the theta rhythm. Cells coding for similar representations are activated together while cells coding for distinct representations are active at different times. (Chapter 3.4.)
5. Switching between two distinct ensemble states occurs within single experimental sessions of the room+arena+ place avoidance. (Chapter 3.5.)

In the discussion (chapter 4.1.) we suggest that the activity of functionally-related groups of hippocampal neurons is organized in time on different timescales. This functional organization may serve to coordinate distinct representations and to prevent interference between them.

1.1. Local and distributed code in the nervous system

The way in which neuronal networks encode information is crucial for understanding the possible mechanism of the coordination of representations. For example, if different mental objects are represented by different, non-overlapping sets of neurons, then multiple representations can be active simultaneously without interference. On the other hand, if different mental objects are represented by distinct activity patterns within the same network, then simultaneous activation of multiple representations would lead to interference and loss of information. This chapter introduces some of the hypotheses of information coding in the nervous system. Compares their properties and discusses experimental evidence supporting different hypotheses.

Local coding

The local coding hypothesis is presented first. It states that every perceived object or thought is represented by the activity of a particular cell (or group of cells) with very specific response properties. The origins of this hypothesis are associated with Horace Barlow (1972) and Jerzy Konorski (1967) who called the cells with finely tuned responses *gnostic units.* According to the hypothesis the representation of an object or an idea in one's mind is equivalent to the activation of a cell that codes for that particular object or idea. The representation of each mental object is localized to a neuron or a group of neurons, representations of different objects is localized to different neurons (Fig. 1). To ridicule the local coding hypothesis Jerome Lettvin coined the term *"grandmother cell theory"*. A grandmother cell is a hypothetical neuron that is activated whenever one sees, hears, or thinks of his or her grandmother. This term is often used today and not always with ironic connotations.

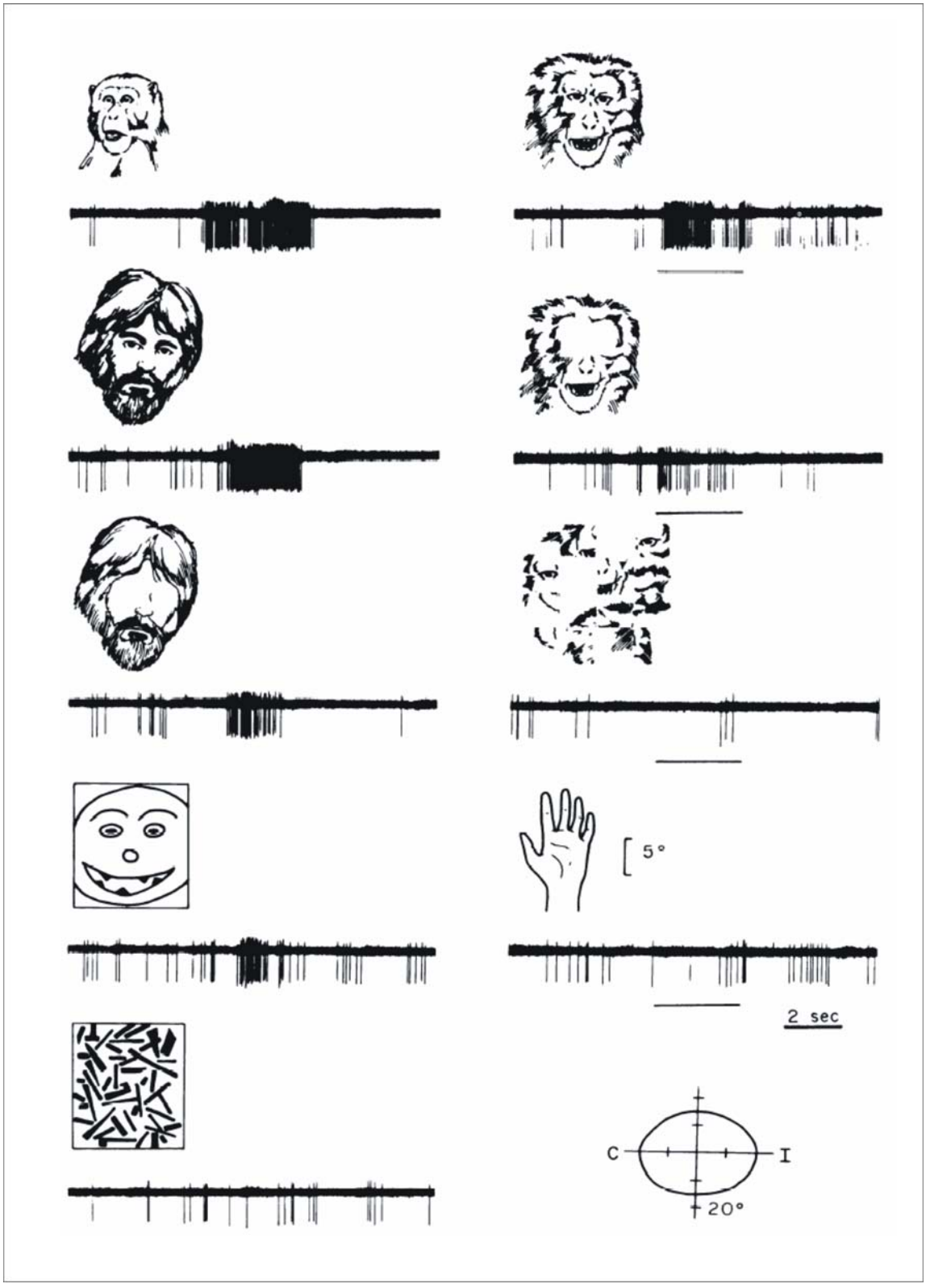

Figure 1. Example of local coding. Discharge of a cell in the superior temporal area of a monkey in response to different visual patterns. This cell responded better to faces than to any other stimulus tested. (From Bruce et al., 1981)

Distributed coding

In contrast to the local coding hypothesis, it has been suggested that information is encoded by the coordinated activity of populations of neurons rather than by the activity of single cells with highly specific responses. This somewhat abstract notion can be introduced by an example of a news display in New York City's Times Square. The display, which delivers short news from world and domestic politics, sports and Wall Street, is composed of thousands of light bulbs. The bulbs are turned on and off in an organized manner so that they form letters and words that can be read by people on the sidewalks. It is not the flickering of any particular bulb that has meaning for readers, it is the coordinated turning on and turning off of large sets of bulbs that delivers a message. If we were observing only one of the bulbs, we would not be able to understand any of the messages. Analogous to this example, the information in the nervous system may be encoded by the coordinated activity of populations of neurons rather than by any particular neuron.

According to this hypothesis a representation of a certain object is distributed across a large population of bulbs (or neurons) therefore this hypothetical coding strategy has been called *distributed coding*.

The idea of distributed coding can be illustrated by a Hopfield network (Hopfield, 1982). One of the questions of cognitive psychology is how nervous system recognizes familiar objects – let's take a dog as an example. There are two problems connected to this seemingly trivial task. On different occasions we see dogs of different breeds, colors and sizes. Even if we see a dog of a breed never seen before, we are able to recognize it as a dog. Therefore different sensory stimuli corresponding to dogs we have seen or may see in the future must all converge to one general representation of a dog. In whatever way the representation of a dog is organized in neuronal networks of our brains, it must have a capacity to generalize. The connected problem arises when we do not see an entire dog but only part of it, the dog can be screened by a tree or a fence for example. Even an incomplete sensory stimulus should lead to a complete representation. A Hopfield network is a model of the nervous system that has both these desired features – a capacity for generalization and a capacity for pattern completion. The Hopfield network is composed of units (analogous to neurons) and connections between these units

(analogous to synapses). The units of a simple Hopfield network shown on figure 2 represent different pixels of a picture; the connections between units are not shown. The network in this example can recognize a schematic picture of a dog and a smiling face. The network completes these patterns after it was presented with distorted or incomplete versions of them. On the left side of figure 2 we see a head of a dog and an incomplete face that were presented to the network. The completed pictures are shown to the right. We will not describe in more detail how the Hopfield network does this task, for our current discussion it is sufficient to emphasize, that in this model it is not the activity of any particular unit (neuron) that encodes a dog or a face. The representation is distributed across the activity of a population of units.

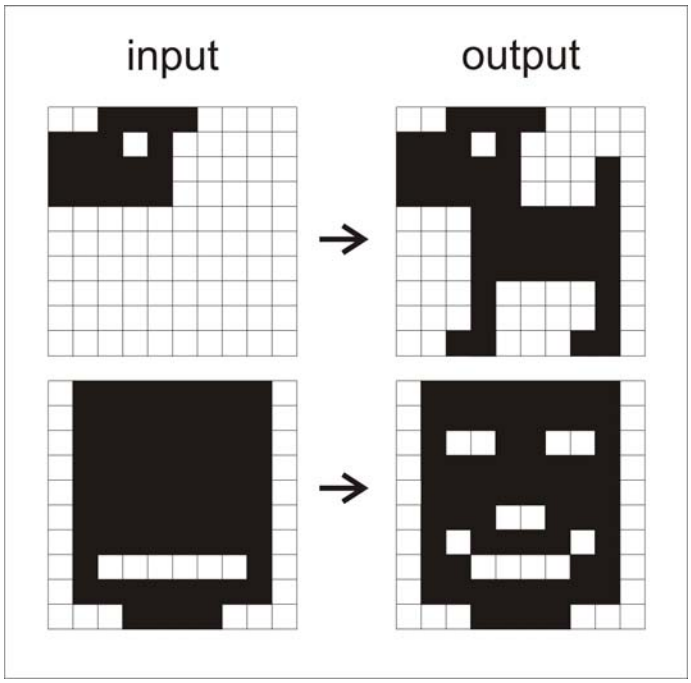

Figure 2. Illustration of distributed coding. In case of a Hopfield network it is not a particular unit ("neuron") that signals the meaning but the population of units. In this example each of 100 units represents a pixel of a picture. The network can recognize a dog and a smiling face (shown on the right side). When presented with incomplete versions of each stimulus (shown on the left), the network was able to complete the patterns.

Cell assemblies, synfire chains

The distributed coding theory was developed in more detail by several scientists. Donald Hebb (1949) suggested that mental objects, "ideas" are encoded by the activity of

groups of neurons he called *cell assemblies*. The cell assembly in Hebb's description is a group of neurons that form a circuitry in which reverberatory activity can be preserved for a short time (less than a second). He suggests that such activity "constitutes the simplest instance of a representative process (image or idea)". In his opinion not all the neurons forming a cell assembly are active at exactly the same time. Some of them are active first, perhaps due to sensory stimulation, these neurons activate another subpopulation of neurons, then the third subpopulation of neurons is activated, etc. At some point such a chain of activation may form a loop by activating the same subpopulation that was active before. The activity within such a loop can then be preserved even in the absence of the stimulus that originally triggered it.

Cell assemblies in Hebb's view are not static objects; they change, and new ones are being created as an animal learns about its environment. Cell assemblies representing parts of a bigger object (Hebb gives the example of the three corners of a triangle) that are often activated in close temporal succession can lead to the creation of another cell assembly representing the whole object (the triangle itself). Once this happens, there are four assemblies that can be activated as an animal looks at a triangle: those representing the three corners, and the one representing the triangle as a whole. As long as the animal looks at the triangle these four assemblies are activated one after another. Hebb calls such a sequence of activation a *phase sequence*. He suggests that a train of thought is in principle also a more complicated phase sequence.

An idea similar to the cell assemblies was elaborated by Abeles (1991). He also suggested that groups of sequentially activated cells are important for information processing, but unlike Hebb, he put emphasis on the exact timing of action potentials emitted by different neurons. In his terminology groups of synchronously firing cells (within 1-2 ms) are called *nodes*. The activity in a node evokes activity in another group of cells – the next node. The set of nodes that sequentially activate each other is called a *synfire chain*.

Comparing the local and distributed information coding hypotheses

For our purposes the most important difference between the local and distributed coding is in the way different representations can be activated simultaneously. In the case

of local coding, different representations can be active at the same time because they are coded for by independent units. In the case of distributed coding the same units participate in the coding of different representations, so only one of the representations can be active at any given moment. This can be illustrated by the analogy of the Time Square display. If we attempted to display two messages simultaneously they would interfere with each other. There are two simple ways to avoid this problem. The different messages can either be separated in space; the display can be divided into several parts that represent different information. Or alternatively different messages can be represented at different times. This solution is used in the case of the Times Square display were sports news alternate with the business information.

Another difference between the two coding strategies is in the way they respond to damage. In the case of local coding, damage to a particular neuron will only affect the corresponding representation but will leave the other representations intact. In the case of distributed coding, damage to a neuron will affect most or all of the representations, but only slightly, most of the information will be preserved. This is analogous to the way damage to one of the light bulbs affects function of the display. The term *graceful degradation* is used for this property of distributed coding.

The third difference between the two encoding strategies is in their capacity. A local coding system can encode at most as many representations as the number of units involved, because each representation requires a separate unit. The maximal number of possible representations in the distributed code is 2^n, if n is number of units available and each unit can be in one of two states.

Which hypothesis is correct?

The question is controversial. A substantial experimental effort aimed at testing different ideas about information coding yielded interesting results. The local coding hypothesis predicts the existence of neurons with highly tuned complex responses. Remarkable experimental data are consistent with this theory, some neurons indeed seem to have very complex and finely tuned responses. In the superior temporal sulcus of a monkey, neurons were detected that were most activated when a face was present in the visual field (Bruce et al., 1981). The activity of such a face sensitive neuron is illustrated

in figure 1. This neuron was firing at the highest rate in response to the face of a monkey or a human face. It was activated less when the monkey looked at a schematic drawing of a face, and it was not activated when the animal was looking at a hand or a random pattern. Similar observations were reported by Quiroga et al. (2005). In the medial temporal lobe of humans they observed cells that were selectively responding to a specific well-known person (Bill Clinton, for example). The cells were activated not only by photographs of the person, but also by drawings, or the written name of the person, therefore they apparently coded for an abstract representation of identity rather than for a particular set of sensory features. Neurons in other parts of the nervous system were also found to have complex, and rather abstract firing correlates. For example, the activity of place cells in the hippocampal formation of a rat correlates with the rat's location in an environment (O'Keefe and Dostrovsky, 1971, Muller et al., 1987), and activity of head direction cells in postsubiculum correlates with the direction the rat's head is facing (Taube et al., 1990a,b).

In spite of the striking responses of neurons as those reported by Quiroga et al. (2005) these data do not give definitive support to the local coding hypothesis. The specificity of a cell's responses is hard to assess because the number of stimuli that can be tested in an experiment is limited. Moreover, the existence of cells with very specific responses by itself does not falsify the distributed coding hypothesis. The distributed coding hypothesis is consistent with finely tuned responses of single cells. This can be illustrated on an example of a very simple representation system – a display that consists of 20 units and codes for digits from 0 to 9 (Fig. 3). If we read the display, we do not look at a particular unit, but at the display as a whole, in this sense the representation of digits is distributed. However, if we look at the behavior of single units, we find that some of them have very specific responses. For example, the unit in the 3rd column of the 2nd row is only active when digit 1 is displayed. Similarly, the unit in the 4th column of the 5th row only activates with digit 2. Although we as readers could use the activity of these single units to identify numbers 1 and 2, we do not do it. Even if these units were not active, we would still identify the corresponding digits based on the information provided by the other units. This example illustrates that although we use a distributed coding strategy to decode (read) the message, some of the units involved have very specific responses.

Figure 3. Specific responses of particular units do not prove that the system is using local coding.
A) A simple system (a display) consisting of 20 units can code digits from 0 to 9. It is the state of the entire display that we use to recognize a digit, in this sense the system is using distributed coding.
B) However, some of the units have highly specific responses, and are only activated when a particular digit is shown. Units that are specific for number 1 or 2 are marked in red.
C) The digits are readable even if these specific units are not active demonstrating that the overall arrangement of the activity is more important for the recognition of digits than the activity of specific units.

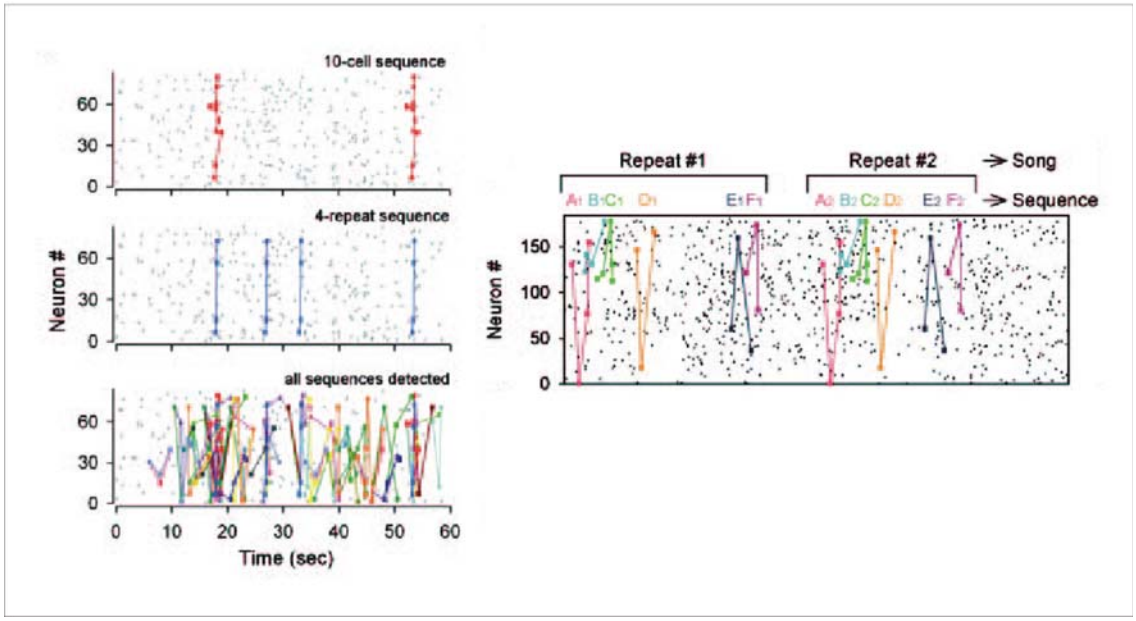

Figure 4. Theory of synfire chains predicts repeated sequences of activation of different neurons. Ikegaya et al. (2004) demonstrated repeated sequential activation of identical cells in a slice of primary visual cortex.
Left: Top figure shows a raster plot of spontaneous activity of multiple simultaneously recorded cells. Repeated sequence of activity of 10 cells is shown in red. This sequence repeated twice during the one minute interval of the data that is shown. The middle plot shows a repeated sequence of activation of four cells, this sequence repeated four times. The bottom plot shows all sequences found in this raster plot. Right: Sequences of activity A, B, C, D, E, and F repeated twice in the same order. The authors coined term "cortical song" for this phenomenon. (From Ikegaya et al., 2004)

When it comes to testing the population coding hypothesis experimentally our tools are limited. Although it now becomes possible to record action potentials from a hundred or so cells simultaneously (Wilson and McNaughton, 1993), this is still only a fraction of the hundreds of thousands of cells that make up a brain region. We may be in a similar situation as if we were looking at a randomly chosen fraction of light bulbs of the Times Square display, and were trying to reconstruct a message from them. Since the available methods do not allow showing directly a cell assembly or a synfire chain, we have to rely on indirect evidence. We may ask what predictions the distributed coding hypotheses make about the behavior of the small subsets of neurons that we can record simultaneously, and we can test these predictions experimentally. For example, if distributed coding was used, then groups of cells that represent a single object should not behave independently, and therefore it should be possible to predict the activity of a particular cell by the activity of other cells in the network. This prediction was tested and confirmed by Harris et al. (2003) in the case of the hippocampal CA1 network.

Abeles's theory of synfire chains predicts that when a synfire chain is activated neurons that participate in it are likely to fire with fixed time delays. Several groups observed that neurons tend to fire with fixed time delay consistent with this hypothesis (Abeles et al., 1993; Mao et al., 2001). Ikegaya et al. (2004) for example reported the existence of characteristic sequences of firing of cortical neurons and called these sequences poetically "cortical songs" (Figure 4).

Inadequate methods to record and analyze the activity of large populations of simultaneously recorded neurons lead to a paradoxical situation. In theoretical discussions when local and distributed coding strategies are compared, arguments for one or the other are weighted; most neuroscientists lean toward the distributed coding. In practical situations, when scientists design their experiments and discuss the data, a strong bias toward the local coding is implicitly present in their decisions. The activity of single cells is usually analyzed, interpreted, and used to assign function to a particular brain region. Experimentally it is much easier to record and analyze the activity of single cells, and look for correlates of their firing.

1.2. Coordinating different representations – the binding problem, superposition catastrophe

This chapter explains the binding problem and superposition catastrophe that arise from simultaneous activation of several distributed representations. Three possible solutions to these problems are discussed: combinatorial (local) coding, attention, and temporal coordination.

The binding problem

Different aspects of a single complex stimulus can be processed independently, often in anatomically distinct brain regions. In the case of visual information processing the dichotomy between the ventral and dorsal stream is an example of such localization of function. Simply stated, the structures of the ventral stream are involved in the recognition of intrinsic object features, while the structures of the dorsal stream process information about the position of objects in space (Ungerleider and Mishkin, 1982). Although different aspects of a perceived object are represented by distinct neural networks in different anatomical locations, they are somehow bound together, so that the object is perceived as a single entity. The task of binding different, independently processed properties of an object together becomes more challenging when more than one object is present in the visual field. This problem was nicely illustrated by Frank Rosenblatt (1961) by an example of a system composed of two pairs of neurons (Fig. 1). Two neurons code for the location of an object; one neuron is activated when an object is detected in the upper part of the visual field, and the other neuron is activated when an object is in the lower part of the visual field. The other two neurons respond according to the shape of an object; they are activated in response to a triangle and a square, respectively. When a square is present in the upper part of the visual field (Fig. 1A) the neuron coding for upper position and the neuron coding for square shape are activated giving unambiguous information about the position and shape of the object. In a more complicated situation, when a square and a triangle are present in the visual field at the same time a problem appears (Fig. 1B). In this case all four neurons are active, and this pattern of activity cannot be interpreted unambiguously. It may be evoked by a square

above a triangle as well as by a triangle above a square. The question of how to disambiguate these two possibilities, how to bind together the neurons representing different properties of the same object, is known as the *binding problem*. Before we proceed to discuss possible solutions to the binding problem we first introduce a related concept – the superposition catastrophe.

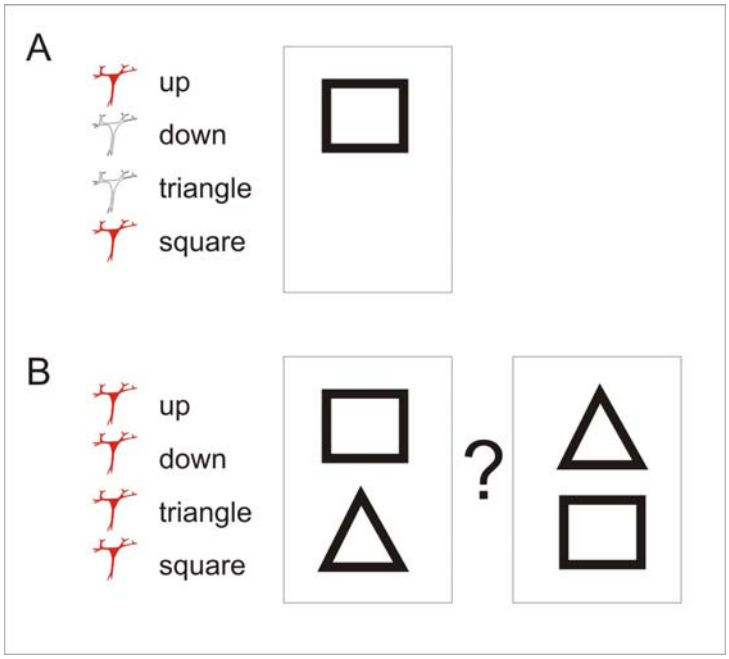

Figure 1. Illustration of the binding problem. The artificial system is composed of four neurons, two of them respond according to the position of an object in the "visual field", and other two according to the shape of the object.
A) A single object – a square – is present in the upper part of the visual field. Neurons coding for upper position and square shape are active (active neurons are shown in red). In this case the activity of neurons can be unambiguously interpreted.
B) If two objects are present in the visual field all four neurons are active. This pattern of activation can be interpreted in two different ways, either as a square above a triangle or as a triangle above a square. (Rosenblatt, 1961)

Superposition catastrophe

If information is represented by groups of neurons rather than single cells, as is the case in distributed coding, then the problem arises of how to activate more than one representation at the same time. As was discussed in the previous chapter, simultaneous activation of more than one representation would cause interference; the information would be degraded or lost. This situation is traditionally referred to as the *superposition catastrophe* (von der Malsburg, 1981, 1999; Milner, 1974)

Several candidate mechanisms have been suggested to solve the two related problems of binding and superposition catastrophe. *Combinatorial (local) coding* mechanisms, *attention*, and *temporal coordination of cell discharge* will be discussed in this chapter. (Issue of Neuron from September 1999, 24(1) contains several reviews devoted to this topic from leading investigators in the field.)

Combinatorial (local) coding

The local coding strategy which was discussed in the previous chapter provides one possible way how to solve the binding problem. According to this hypothesis there is a specific neuron for any possible combination of features, and activation of this neuron is equivalent to a particular representation. In the Rosenblatt's example (Fig. 1) the problem could be solved by existence of neurons that would respond to specific combinations of position and shape. There could be a neuron that would fire when a square is in the upper position, etc. The main problem with this solution is the overwhelming number of possible combinations of features that the brain can be confronted with, a situation called "combinatorial explosion". The unreasonably large number of neurons required makes combinatorial coding an unlikely candidate for a universal solution to the binding problem. However, combinatorial coding has been shown to be sufficient to deal with some problems in visual information processing (Riesenhuber and Poggio, 1999).

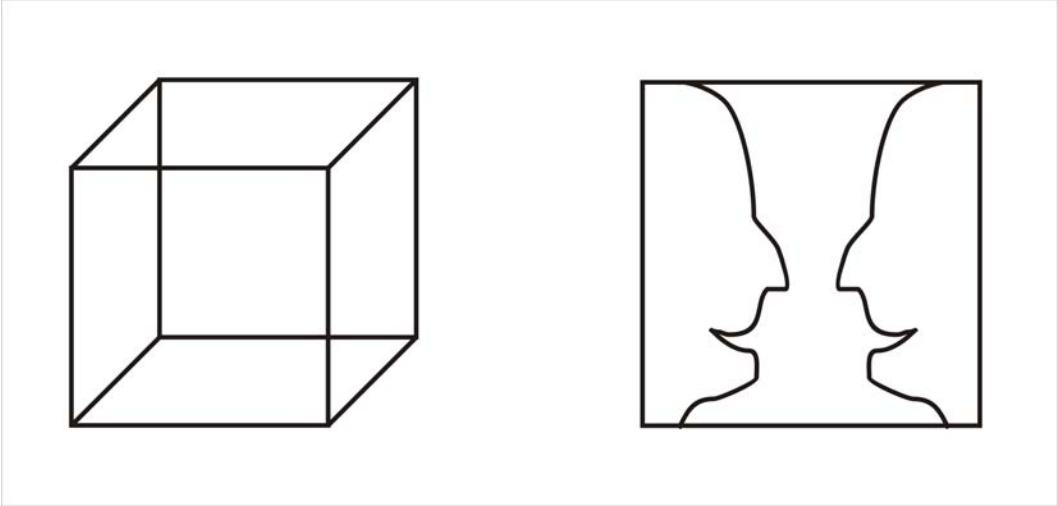

Figure 2. Examples of bistable images. Necker cube on the left can be seen as being viewed from above or from below. When one is watching the drawing for a while, the perception spontaneously switches between these two interpretations. Similarly the drawing on the right can be interpreted in two ways – as a vase or as profiles of two heads facing each other.

Attention

The other two mechanisms suggested to solve the binding problem are attention and temporal coordination. They share a common underlying idea – that only one representation is active at any given time. Introspection and psychological experiments show that when presented with an ambiguous sensory input that allows several interpretations, only one of the competing interpretations is active at any moment (Fig. 2). Many psychophysical experiments also showed that attention helps to solve tasks, which require binding different properties of objects together (Wolfe and Cave, 1999).
There is neurophysiological evidence that activity of single cells in the visual areas can be influenced by attention. Experiments of Reynolds and Desimone (Reynolds et al. 1999) suggest that if attention is focused on one object within the receptive field of a V2 neuron, the cell's response is dominated by this object, even if other "distracting" objects are present in the visual field simultaneously (Fig. 3). This is consistent with the hypothesis that attention biases the neuronal representation toward one object and suppresses the representation of alternative objects. So that the cause of the binding problem – several representations present at the same time – can be avoided.

Temporal coordination

An alternative mechanism that would allow competing representations to be disambiguated relies on synchronizing neurons that code for the same represented object on a timescale of a few milliseconds (Milner, 1974; von der Malsburg, 1981, 1999; Gray, 1999). It has been suggested that high frequency oscillations in the beta and gamma range could serve as a pacemaker for such synchronization. Consistent with this hypothesis Engel et al. (1991) reported that the activity of neurons that code for the same object in the visual field is synchronized (at the time-scale of gamma oscillations) while the activity of neurons coding for different simultaneously present objects is not (Fig. 4). They recorded multiunit activity from two locations in the cat primary visual cortex. The responses at the two recording sites were optimally correlated with the presence of a light bar in two different orientations. These preferred orientations are shown by the green and red bars in figure 4. In the first experiment, only one moving bar was projected to the visual field, and its orientation was between the optimal orientations for the two

recording sites (Fig. 4A). In these conditions the activity at the two recording sites was correlated. The peak of the cross-correlation at 0 milliseconds suggests a tendency of the neurons to fire at the same time. The other peaks approximately 20 ms from the first one suggest a synchronized oscillatory activity at the two recording sites. The frequency of the oscillation is around 50 Hz, which corresponds to the gamma rhythm prominent in the neocortex. In the second experiment two bars were present in the visual field at the same time (Fig. 4B). Each bar had the orientation optimal for one of the two recording sites. Under these circumstances the firing at the two recording locations was not correlated. This observation is consistent with the hypothesis that if different cells are coding for the same object (the same bar in the first experiment) their activity should be correlated, but when the cells are coding for different objects (two different bars in experiment 2) they should not have correlated activity.

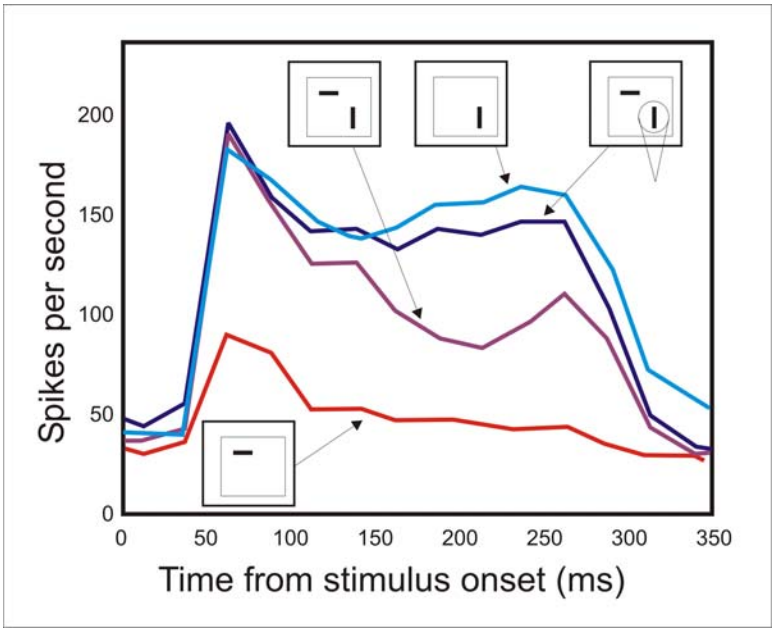

Figure 3. Attentional modulation of single cell responses in area V2 in macaque.
If attention was focused outside the receptive field (thin-lined square) of the neuron and the two stimuli were present separately, they evoked different responses. The neuron responded strongly when the vertical line was present alone (light blue line) and the response was weak when the horizontal line was present alone (red line). Both stimuli presented simultaneously caused intermediate response of the neuron, if attention was focused outside the neuron's receptive field (brown line). If both stimuli were present and attention was focused on one of them, then the cell responded as if this was the only stimulus present (dark blue). The not-attended stimulus was "filtered out".
This experiment provides evidence that a single neuron can respond to a single stimulus at a time, and that attention can control which of the potential stimuli are being processed by the neuron. (From Reynolds and Desimone, 1999)

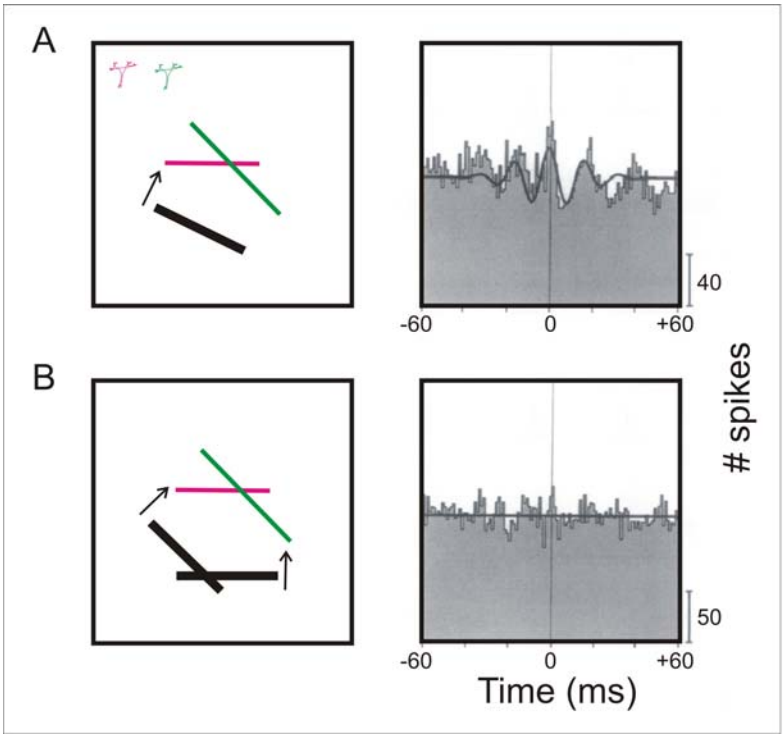

Figure 4. Neural activity was recorded from two different locations in the primary visual cortex of a cat. Neurons in one location responded strongest when horizontal bar (red line) was moving through the visual field. Neurons at the other recording location had the strongest response to the bar rotated by 45˚ (green line). On the left side of the figure an experimental conditions are schematically represented. The red and green lines represent the preferred orientation of a bar for the two recording locations. The thick black lines and arrows show the orientation and direction of movement of a bar (or bars) that was presented during an experiment. On the right side the cross-correlation between the activity at the two recording sites is shown.
A) In the first experiment one moving bar was projected to the visual field. The orientation of the bar was in the middle between the preferred orientations of the two recording sites. Under these conditions the activity at both recording sites was correlated.
B) In the second experiment two bars with different orientations (optimalized for the two recording sites) were moving through the visual field simultaneously. Under these conditions the activity at the two sites was not correlated.
These experiments show that activity of cells was synchronized when they responded to the same object, but the activity of neurons was desynchronized, when they were responding to different simultaneously present objects. (From Engel et al., 1991)

Experimental data from the visual system support the notion that the activity of cells coding for the same object is synchronized and activity of cells coding for different objects is separated in time. This can be achieved by attention or temporal coordination and for both of these strategies exists some experimental evidence. The suggested mechanisms can work simultaneously and serve different roles in visual information processing. For example, during the pre-attentive stage of processing the temporal coordination (working at millisecond time scale) can be used to identify “candidate”

objects based on “Gestalt” criteria like spatial proximity, continuity, or similarity in movement. These candidate objects can then be closely analyzed using attention based mechanisms that operate at time scale of seconds (Wolfe and Cave, 1999).

Although the problems of binding and the superposition catastrophe were predominantly studied in the visual areas, very similar problems must be solved by other brain systems. The theoretical and experimental work done in the visual system may provide useful clues about the organization of the hippocampal representations of space.

1.3. Place cells in the hippocampus

This chapter introduces the traditional classification of hippocampal neurons into two categories: complex-spike cells and theta cells. It discussed spatial responses of hippocampal neurons. Place cell responses to "space" and not merely to sensory stimuli are described. The lack of simple topographical organization of place cells within the hippocampus is discussed. Finally, the notion of change of hippocampal representation between different contexts (remapping) is introduced.

Complex-spike cells, theta cells

Early recordings of hippocampal single cells in awake, freely-behaving rats revealed two distinct classes of neurons. Based on the firing rate, the waveform of extracellularly recorded action potentials, and the temporal organization of firing, the hippocampal units were classified as *complex-spike cells* and *theta cells* (Ranck, 1973; O'Keefe and Conway, 1978). Complex-spike cells have broader action potentials, fire at low average rates, rarely higher than one spike per second, and sometimes discharge in bursts of two to five action potentials approximately five milliseconds apart – so called complex spikes. The amplitude of subsequent extracellular action potentials within a complex spike often decreases. Theta cells have narrow action potentials, higher average firing rate (around thirty spikes per second) and do not fire in bursts. The firing rate of theta cells increases when oscillatory activity of 6 -10 Hz (theta rhythm) is present in the hippocampal field potential recording (EEG). Electrophysiological studies have shown that complex-spike cells are projection cells and theta cells are most likely interneurons (Fox and Ranck, 1981).

Spatial activity of hippocampal units

In the first recordings from freely behaving rats, different kinds of behavioral correlates of hippocampal single cell activity were observed. Some cells fired when a rat was at certain locations of an environment (O'Keefe and Dostrovsky, 1971), other cells were activated when the rat was approaching a reward, or when the rat was exploring a place where it expected a reward but the reward was missing, and still other cells

responded to an experimenter's hand, or to stimuli as bizarre as the presence of a rubber crocodile at a certain location (O'Keefe and Conway, 1978). In spite of the variability in cells' responses in these original experiments, very soon the spatial correlates of the hippocampal pyramidal cells' firing attracted the most attention and shaped the mainstream thinking about hippocampal function. This was reinforced by an influential book "Hippocampus as a Cognitive Map" by O'Keefe and Nadel (1978). Based on an extensive review of behavioral and electrophysiological data, the authors argued that the function of the hippocampus is to represent locations in an environment and spatial relations between them. They proposed that this representation is used to solve spatial problems and navigate through space. Since then the cognitive map paradigm has dominated the way the scientific community thought about rodent hippocampal function.

The two classes of hippocampal neurons differ in their spatial activity as is illustrated in figure 1. The left part of figure 1 shows the trajectory of a rat during a 20 minute recording session in gray, and places where action potentials of a cell occurred in red. The middle panel shows the activity of the same cell during the same session in a form of a firing rate map. The arena was divided into pixels of 2.5x2.5 cm, and the average firing rate of the cell was computed during the time the rat spent in each pixel. The complex spike cell in figure 1 was active when the rat was in the "west" part of the circular arena. Cells with this characteristic were named *place cells* (O'Keefe and Dostrovsky, 1971), and the location (or locations) of the environment where a place cell fires is called a *firing field* or a *place field*. Theta cells often discharge everywhere in an environment, and although their activity can be spatially organized, it is not restricted to a small and discrete firing field (Kubie et al., 1990).

What do the place cells respond to?

Are place cells responding to a certain landmark on the floor, a visual scene, or a more abstract notion of space? O'Keefe and Conway (1978) observed that the firing of most of the place cells is not affected by removal of any particular landmark. This reinforced the notion that the place cells are not merely high-level sensory neurons but rather they form an abstract representation of space. The stability of the hippocampal

representation in face of small changes in the environment would be desirable if the representation was to be used for navigation in a familiar, but changing world.

The role of landmarks in controlling the spatial firing of place cells was thoroughly studied by Muller and Kubie (1987). In accord with the leading cognitive map paradigm, their experiments were intentionally designed in a way that would allow to study the spatial responses of place cells and minimize the influence of other factors such as the rat's behavior or the presence of a reward. They used a simple circular environment with one prominent landmark – a white sheet of paper (cue card) posted on the gray wall of a cylinder. The rats were reinforced to walk around for food pellets scattered to random locations on the floor of the cylinder. This way the experimentalists ensured that the walking and eating behavior was spread evenly on the arena surface, so that the differences in cell's firing in different places of the arena could be unambiguously attributed to the rat's position.

If the position of the paper cue card was rotated 90º, the firing field of a cell rotated together with it, so that its position relative to the cue was unchanged. This suggested that the prominent landmark has control over cells' firing (Muller and Kubie, 1987). When the paper was removed from the wall altogether, the cell continued to fire at the same physical location as it used to fire in the presence of the paper (Muller and Kubie, 1987). The locations of the cells' firing fields did not change even if the light was turned off during an experimental session so that the visual landmarks were eliminated (Quirk et al., 1990). These experiments showed that although the visual landmark had a strong control over the spatial responses of cells, the presence of a single salient cue was not necessary for the firing, and in its absence other cues controlled the cell's activity.

Sharp et al., (1990) addressed the role of visual landmarks in hippocampal coding for space by studying place cell responses in a visually symmetrical environment. They observed that place cells fire differently when the rat is in two distinct but visually equivalent locations. This result again shows that place cells do not respond merely to sensory stimuli but rather to spatial location as is predicted by the cognitive map hypothesis.

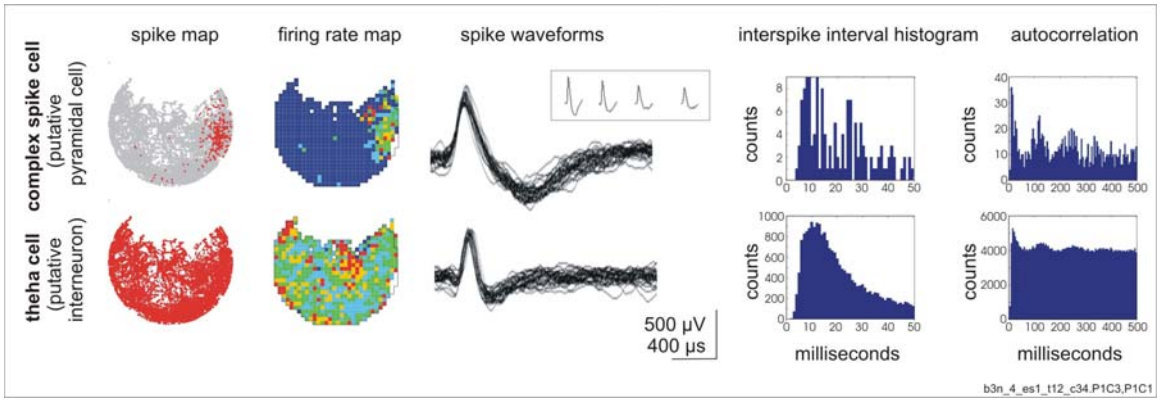

Figure 1. A complex-spike cell and a theta cell. In the top row activity of a complex spike cell is shown, in the bottom row a theta cell is characterized. The cells were recorded while the rat was foraging for pellets on a circular arena. The rat was reinforced (by a mild foot shock) to avoid a zone at the north part of the arena, therefore this part of the arena was not visited by the rat. In the left picture the trajectory of a rat during the recording session is shown in gray and locations where the cells fired action potentials are shown by red dots.
The second panel from left shows the spatial distribution of the firing of the same cells in a different way. The arena was divided into pixels 2.5*2.5 cm and for each pixel an average firing rate of the cell was computed. (The number of spikes emitted in each pixel was divided by the time the rat spent in that pixel.) In the firing rate map of the complex-spike cell the red color corresponds to 7.0 or more action potentials per second and dark blue to 0 action potentials per second. For the theta cell the red color corresponds to 43.2 or more action potentials per second.
In the middle of the figure 30 superimposed waveforms of the action potentials are shown. Notice shorter duration of action potential of the theta cell compared with the complex-spike cell. In the inset above waveform of the complex-spike cell is a typical example of a complex spike; a sequence of action potentials with decreasing amplitude. The spike waveforms in the inset show 2 ms of data.
The plots on the right show the interspike interval histograms and the autocorrelation of two categories of cells. The interspike histogram of the complex spike cell shows a peak at values below 10 ms. These are the intervals between subsequent action potentials within a complex spike. In the case of the theta cell this peak is missing – the cell did not discharge in complex spikes.
The autocorrelation plots of both cell types have a peak at approximately 120 ms. This corresponds to the period of the hippocampal theta rhythm. Discharge of both cells was modulated by theta.

Is the place cell representation of an environment topographically organized?

In many cortical regions, cells with similar responses are anatomically clustered together. This can be observed in sensory areas such as visual (Hubel and Wiesel, 1962) or somatosensory cortex (Mountcastle, 1957). Early on it was noticed, that hippocampal place cells recorded from nearby anatomical locations could have firing fields distributed throughout an environment (O'Keefe and Conway, 1978; O'Keefe, 1979). This can be illustrated by an example of the recording on Figure 2. The figure shows the spatial distribution of discharge of eight place cells recorded simultaneously. The cells 1 to 4 were recorded from four electrodes directly attached to each other (a tetrode, discussed in more detail in chapter 2.2.), therefore one can assume that the cells were very close to

each other in the hippocampus. The firing fields of these anatomically neighboring cells are distributed across different locations of the environment. Cells 5 to 8 were recorded from a different tetrode that was implanted at least 400 μm from the first one. The firing fields of some of these cells overlap with the firing fields of the cells recorded with the first tetrode. For example, the firing fields of cell 1 and cell 5 are at similar places and partially overlap. These data illustrate that neighboring neurons do not always code for neighboring places, there is no simple topographical mapping between locations in an environment and the hippocampal representation of those locations.

However, the question of a possible topographical organization of hippocampal responses remains controversial. Eichenbaum and colleagues reported that firing fields of neighboring place cells overlap more than would be expected by chance (Eichenbaum et al., 1989). Additional evidence suggesting a possible topographical organization in the hippocampus comes from experiments with rats during a delayed-nonmatch-to-sample task (Hampson et al., 1999). The cells coding for different position (left or right), and for different phase of the task (sample or non-match phase) were organized in 600-800 μm segments along the long hippocampal axis.

Although the most thorough study of possible co-localization of neurons with similar response properties in the hippocampus included over 3000 neurons, and did not find evidence for clustering of responses in anatomically adjacent neurons (Redish et al., 2001) this topic still remains a subject of passionate debates (Hampson et al., 2002).

Different representations – remapping

In each standard experimental environment approximately 30-40% of complex-spike CA1 cells are active (Wilson and McNaughton, 1993; Vazdarjanova and Guzowski, 2004). If a rat is placed into two different familiar environments – different groups of place cells are activated (Muller and Kubie, 1987; Lever et al., 2002; Wills et al., 2005; Leutgeb et al., 2005). The representations of two different environments are independent. If a cell is active in one environment, it may be, but does not have to be active in another environment. If two cells happen to be active in two different environments the relative position of their firing fields can be different in the two environments. In one environment the firing fields may be overlapping, while in the other environment the

firing fields may be far apart. Consistent with the tradition of calling hippocampal representation of an environment a "map", the phenomenon of having different representations in different environments is referred to as a *remapping*. The remapping can be induced within the same environment when a rat is exposed to two different tasks (Markus et al., 1995). This shows that it is not just the location relative to the landmarks that the cells are responding to but also the "behavioral context" of the task.

It takes a few minutes to build a map of a space that is encountered for the first time (Wilson and McNaughton, 1993). However, a map once built may be changed as the rat gets more experience with an environment. Building distinct representations of two similar but distinct environments may take a few weeks (Lever et al., 2002).

The hippocampal representation of space has been thoroughly characterized through decades of experimental effort. Building on this knowledge several models of hippocampal function have been developed. They will be presented in the next chapter.

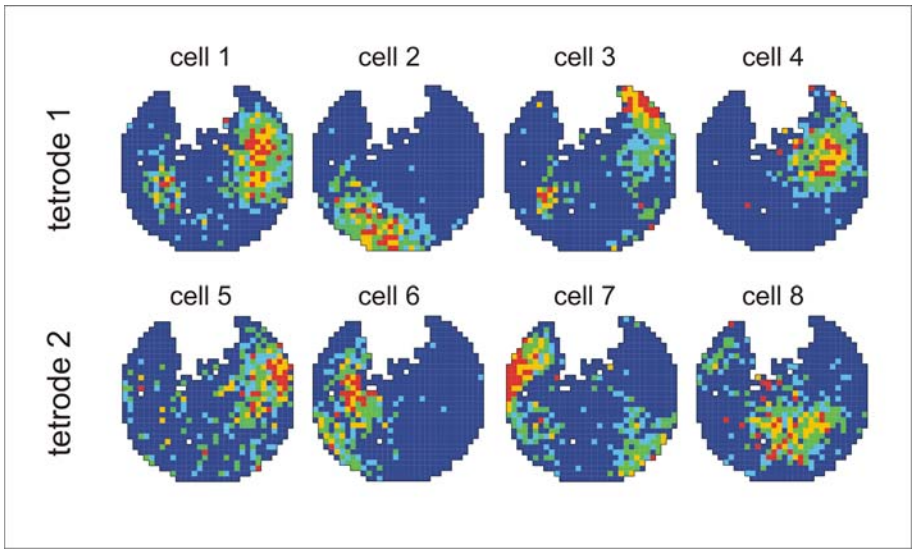

Figure 2. Firing rate maps of eight simultaneously recorded complex spike cells. Cells 1, 2, 3 and 4 were recorded from one hippocampal location by the same tetrode, cells 5, 6, 7 and 8 were recorded from a different hippocampal location at least 400 micrometers away by a different tetrode. The picture shows that some of the cells that were anatomically close responded to very different locations (cells 2 and 4 for example), while some cells that were anatomical separated had very similar responses (cells 1 and 5). This suggests that there is no simple topographic organization of the hippocampal representation of space.

1.4. From cognitive map to attractor network and beyond

This chapter introduces theoretical models of the organization of spatial representation in the hippocampal network. Special attention is devoted to the most detailed and influential attractor network model. Experiments testing the predictions of the theoretical models are also described.

The cognitive map theory of O'Keefe and Nadel (1978) was based on two main ideas. First, the authors argued, that the allocentric space (space that is defined independently of a subject) is represented in the brain. Second, they suggested that this map-like representation is located in the hippocampus. Only a limited knowledge of the properties of hippocampal neurons and functional organization of the hippocampal network was available at the time. This fact prevented the authors to propose a detailed model of the neuronal substrate underlying the cognitive map, although they offered some speculations on this topic. A detailed and very influential neuronal model that could accommodate the cognitive map was developed later and is known as an *attractor network model* (Samsonovich & McNaughton, 1997; Redish 1999).

Hippocampus as an attractor network

To set the stage for discussion of this model, it is useful to clarify the concept of an attractor first. An attractor is a stable state of a dynamical system, which has special properties. A system as simple as a ball on a bumpy surface can serve as an example of attractor properties (Fig. 1A). The state of this system is defined by the position of the ball on the surface. If the ball is on the top of a hill, this system is in one state, if the ball is in a valley, the system is in a different state, etc. There are as many possible states as there are possible positions of the ball. Some of these states have special properties. If we put the ball on an arbitrary location and let it move under gravity, the ball will roll into one of the valleys and stay there. The state of the system when the ball is in a valley is an *attractor state*. The system tends to reach this state spontaneously, it is "attracted" to it, and once it reaches it, the system will not change its state spontaneously, but only in response to a force from outside.

According to the attractor network hypothesis of hippocampal function the synaptic matrix between hippocampal neurons shapes the hippocampal network behavior in such a way, that at any given moment only subpopulation of principal cells is activated, while most other cells remain silent (Fig. 1C). These properties are mediated by sparse excitatory connections between cells that are active together, and global inhibition that ensures that most of the cells are silent most of the time (Fig. 1B). The excitation can be mediated by collateral connections that exist between pyramidal cells of CA1 and especially the CA3 hippocampal subregion (Shepherd, 1998). The inhibition can be mediated by inhibitory interneurons (Freund and Buzsáki, 1996). In figures 1B and 1C the neurons with strong excitatory connections are depicted close together for convenience, the theory however does not assume that cells with excitatory connections are anatomically clustered.

The network organized this way has one important property. If randomly chosen neurons are activated and the network is then left without external input, after some time the system reaches a state, in which a group of neighboring neurons are active and all the other neurons are inhibited (Samsonovich & McNaughton 1997; McNaughton et al., 1996; Redish 1999; Fig. 1C). Each time the network is stimulated with random initial activity pattern, it reaches a state with a different group of neighboring neurons activated. These states are called attractor states, because the system has a tendency to reach them from random initial conditions, and once these states are reached the system does not abandon them without external input. (This is analogous to the ball rolling into the valley and staying there.)

According to the original formulation of the model, the attractor properties are built into the hippocampal network, and are independent of external sensory inputs. The transition between different attractor states is achieved by inputs from self-motion information. Only after exposure to an environment, each attractor state of the network is associated with the sensory cues from the environment that are characteristic for a particular location.

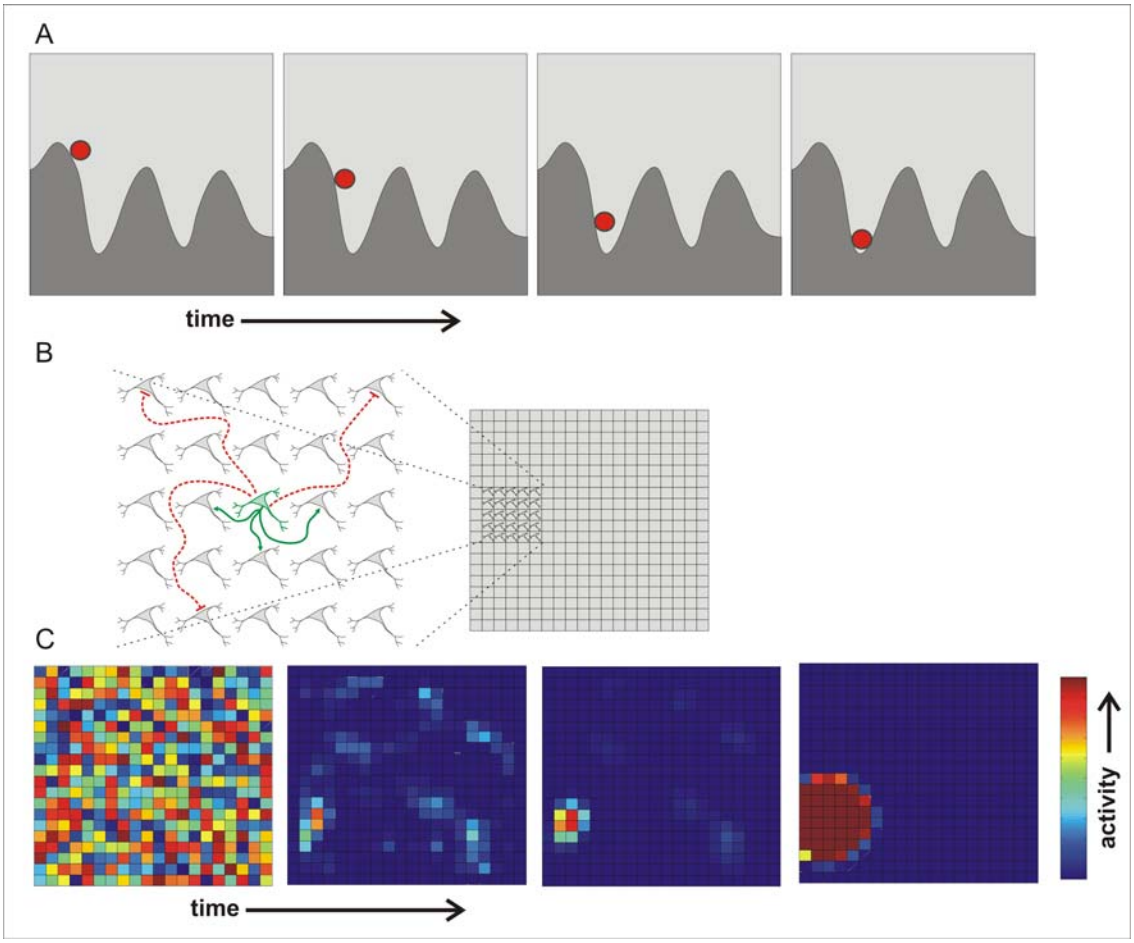

Figure 1. Attractor network. A) A schematic drawing of a ball (red) on a bumpy surface (dark gray). When the ball is released at a random location (left diagram) it rolls down the hill and stays there. When the ball is in a valley the system is in an attractor state (right diagram).
B) Schematic drawing of a distribution of synaptic connections in an attractor network with local excitation and global inhibition. Twenty-five neurons are shown, excitatory synapses are represented by green lines and inhibitory effects are shown in red. Neurons close to each other are connected via excitatory synapses and neurons further apart are mutually inhibited through the action of interneurons (interneurons are not shown in this simplified drawing). Although place cells with nearby firing fields can be located in different locations within the hippocampus, in the figure B and C the cells are drawn in such a way, that the cells with neighboring firing fields appear next to each other.
C) Behavior of a simulated neural network with local excitation and global inhibition. Each neuron is represented by a square; the color of each square indicates the level of activation of a particular neuron. When the neurons are randomly activated (left diagram) the network activity will spontaneously organize in such a way that a group of nearby neurons will stay activated and all the other neurons will be inhibited (right diagram). Once the network reaches such state, it will not change further without additional input – it is in a stable attractor state.

If the hippocampal network worked this way, it would provide an excellent substrate for the hypothesized cognitive map. It would provide a means for coding locations, as well as the spatial relations between them. Each attractor state could be associated with a certain location of an environment. Similar (neighboring) locations

would be coded for by attractor states with high overlap of active units, very different locations would be represented by attractor states that share only few active units. The system could move relatively easily between similar attractor states coding for neighboring locations in an environment (Redish, 1999), but it would be unlikely to switch abruptly between states representing distant locations.

In the models of Samsonowich and McNaughton (1997), and Redish (1999) the dynamics of an attractor network is primarily controlled by self-motion cues internal to the rat, and secondarily by visual and other landmarks of the environment surrounding the rat. After the rat is introduced to the familiar environment, the sensory stimuli activate the pattern of activity appropriate for the environment and the rat's location in it. After the appropriate attractor state was set, it could be changed merely by the self-motion input. This way the spatially-selective activity of cells can be preserved even in the absence of environmental landmarks, for example in darkness as was observed by Quirk et al. (1990). The model is also consistent with the fact that place cells can be controlled by self-motion cues for few seconds before the external visual landmarks take control over them (Gothard et al., 1996; Redish et al., 2000; Rosenzweig et al., 2003). Since there is an inherent error in the rat's self-motion based navigation (Stuchlík et al., 2001), from time to time, the activity of a network has to be updated to match external landmarks.

The attractor network hypothesis of hippocampal function implies distributed coding of information. Neurons are not responding independently of each other. The activity of each neuron is closely related to the activity of its peers.

Independent feature detectors

In one of the first papers that characterized spatial properties of place cells, O'Keefe and Conway (1978) studied the responses of place cells when some of the landmarks were removed from an experimental environment. Although they found that the activity of most of the cells did not depend on any single landmark, some cells seemed to be controlled by one or two landmarks and ceased firing when those landmarks were removed.

The results of Shapiro et al. (1997) also indicated that different place cells can respond independently to different subsets of landmarks. Their experiment was

performed on a maze with four arms extending from a central platform in four different directions (plus maze). In the apparatus, multiple landmarks were available for orientation, some of them were local – tactile cues on the floor of each arm; the others were distal – visual cues around the apparatus. By systematically re-arranging the landmarks or removing some of them, the experimenters were trying to determine which subsets of landmarks control each of the place cells. They observed that some cells responded in relation to an individual distal or local cue, some responded to the relationship among several cues within a stimulus set – either local or distal – and others to the relationship between distal and local cues. These experiments provide evidence for the notion, that cells can be controlled independently and simultaneously by different subsets of sensory stimuli. We will call such hypothetical cells *independent feature detectors*.

Testing the attractor network hypothesis

To test whether the place cells respond as a cohesive attractor network or as independent feature detectors several groups performed "double rotation experiments" with somewhat similar design (Fig. 2). Two subsets of landmarks were manipulated independently between two recording sessions and responses of the place cells to this manipulation were studied (Tanila et al., 1997; Brown and Skaggs, 2002; Knierim, 2002; Fenton et al., 2000). First, place cell responses were recorded during a "standard session". Then one subset of landmarks was moved clockwise, the other subset counter-clockwise. After this manipulation the same cells were recorded during the second recording session called a "double rotation session". If the attractor network hypothesis is correct then the hippocampus as a unit represents spatial locations and therefore all of the simultaneously recorded cells should behave cohesively during the double rotation session. All cells should follow the same "rule" in changing their response. Their firing fields could rotate together with one subset of landmarks ignoring the other one, or alternatively, the cells could respond to both sets of landmarks cohesively in some more complicated way, or they could change their responses completely. If the cells are responding independently, then some of the simultaneously recorded cells might follow one set of landmarks while other cells could follow the other set of landmarks.

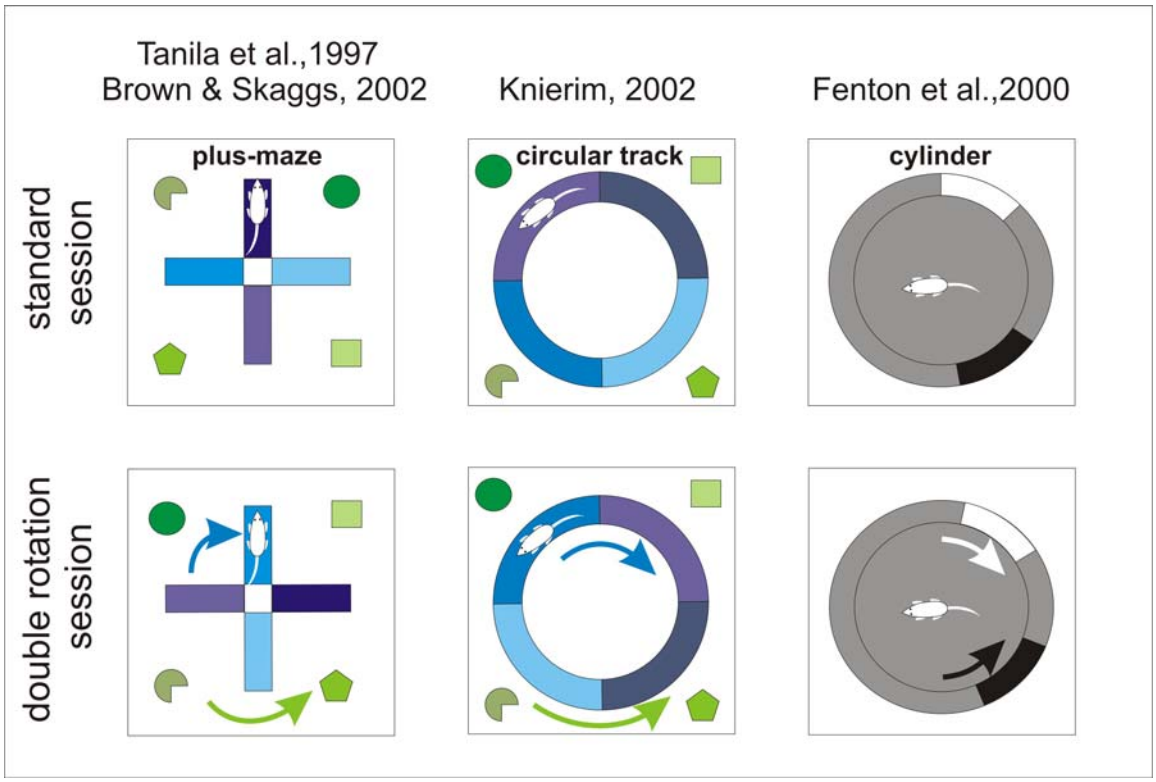

Figure 2. Schematic outline of double rotation experiments performed by different groups. In all of these experiments place cells were recorded when two sets of landmarks were independently rotated in opposite directions between two recording sessions. The session before cue rotation is called a standard session, the session after rotation is called a double rotation session. Experiments of Tanila et al. (1997) and Brown and Skaggs (2002) were performed in a plus maze, and the two independently rotated sets of cues were local landmarks (blue) and distal landmarks (green). Knierim (2002) used a circular track, and he also rotated local (blue) and distal (red) cues independently. Fenton et al. (2000) exposed the rat to two equally salient paper cue-cards (black and white) attached to the gray wall of a cylinder, and moved these cue cards in opposite directions.

The double rotation experiments performed by different groups did not lead to a consensus on this topic. A clear tendency for most of the simultaneously recorded cells to follow the same set of landmarks was observed, as would be predicted by an attractor network hypothesis. At the same time not all the cells behaved cohesively, which supports the notion of independently acting feature detector units. A different outcome came from an experiment by Fenton et al. (2000). They showed that moving two equally salient landmarks apart or together resulted in stretching or squeezing of the existing hippocampal representation of space. In their hands, a firing field of a particular cell did not follow one or the other landmark, but influences of the two landmarks were

"averaged". The place cell response changed according to relative distances between its firing field location and the two landmarks.

Another convincing demonstration of hippocampal pyramidal cells being independently controlled by different subsets of available stimuli came from Rivard et al. (2004). If a transparent barrier was moved from one place to another in the same environment, some cells responded to the animal's position in the enclosure. Other cells, those that happened to discharge close to the barrier, followed the barrier responding to the animal's position relative to the barrier. This shows that some cells can be controlled by salient landmarks regardless of the rat's position in an environment, independently of other cells that respond according to the rat's position.

Maplets

A large body of evidence seems to contradict the hypothesized cohesive attractor dynamics of the hippocampal network. Under some circumstances different cells may be controlled independently within a single recording session. To explain these data and reconcile them with the elegant attractor network hypothesis Touretzky and Muller (2006) proposed the existence of *maplets*. Maplets are independent functional sub-networks working in parallel within the hippocampus. Each of the maplets is organized as an attractor network, and can be controlled by a different subset of environmental landmarks. In the paradigm used by Rivard et al. (2004) one maplet can be controlled by the transparent barrier, while a different maplet can be controlled by the distal environmental cues.

Changes of representations within a session

Another way to explain the existence of different responses from simultaneously recorded cells would be to expect that different representations are active at different times. For some time the network could be in one state, responding according to one set of landmarks, and at a different time within the same session it could respond to a different set of landmarks. This would explain the inconsistent responses among cells recorded simultaneously within the same session, and at the same time would be consistent with the attractor network hypothesis. Several reports indicated changes in

hippocampal representations within a session. Sometimes a change of cells' responses within a session can be explained by a particular feature of an experimental design. This was the case for experiments in which place cells were recorded while a rat was running on a linear track, between a start box and a goal (Gothard et al., 1996; Rosenzweig et al., 2003; Redish et al., 2000). The position of a start box relative to the track was changed between trials. At the beginning of a trial the place cells responded according to the rat's position relative to the start box. A few seconds later in the trial the cell responses "switched" and followed the rat's position within a room.

Even if the behavioral contingencies do not change, the responses of place cells may be extremely variable within a single session. Fenton and Muller (1996) reported that during different passes through a firing field of a place cell the discharge of the cell varied more than would be predicted by variability of a Poison process. The observed variability in cells' discharge could be expected, if the cell's firing was controlled by two different processes at different times, and these two processes would switch at a timescale of seconds (Olypher et al., 2002). Recently more evidence for switching between two hippocampal representations within a single task was reported (Jackson and Redish, 2007; Lytton et al., 2007; Kelemen and Fenton, 2007).

Cell assemblies

A different form of temporal organization of hippocampal representations was suggested by Harris et al. (2003). They proposed that groups of hippocampal place cells form "coalitions" that fire in close synchrony (Fig. 2). The term *cell assemblies* was borrowed from Donald Hebb to refer to these coalitions of cells. (Chapter 1.1. contains a discussion of Hebb's cell assembly theory.) In an effort to test their hypothesis experimentally Harris et al. (2003) recorded the activity of large ensembles of place cells simultaneously. They reasoned that if the cells are indeed organized into assemblies, then activity of a particular cell should be closely related to activity of other cells that are members of the same cell assembly. Consistent with this expectation they observed that if activity of the other simultaneously recorded neurons is taken into account, the activity of a particular place cell can be predicted more accurately and this prediction is most accurate if computed at time-scale of hippocampal gamma oscillations (10-30 ms).

How do these models relate to our central question of coordinating different simultaneously relevant representations? If the hippocampal cells are independent feature detectors, then they can independently and in parallel code for the two distinct representations. Independently working sub-networks, suggested by maplet hypothesis, could also process information about different representations in parallel. If however, the whole hippocampus is organized as a single attractor network, then all the simultaneously recorded neurons should respond to the same reference frame. The cell assembly model would predict that different representations can be active within the same experimental session, but should be separately organized in time.

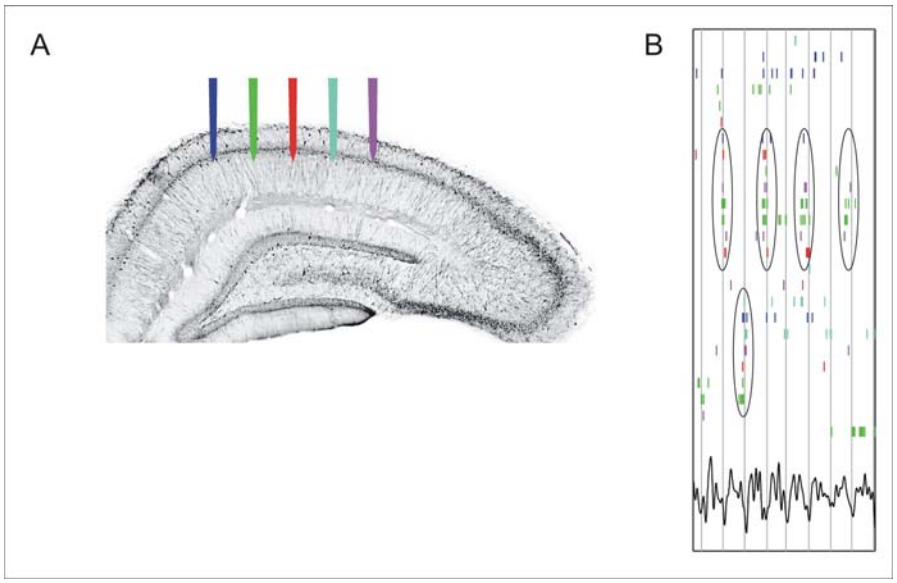

Figure 3. Illustration of cell assemblies in hippocampal network as presented by Harris et al. (2003). A) Activity of pyramidal cells was recorded from five different locations of the CA1 hippocampal subregion. B) Raster plot of one second of data showing activity of 25 simultaneously recorded cells and local field potentials with prominent theta rhythm. Two groups of cells with synchronized firing are marked by ellipses. The cells marked this way are participating in a putative cell assembly. (From Harris et al., 2003)

1.5. Coordinating different spatial representations and a role of the hippocampus in such coordination

Continuous rotation of the arena in the room allows dissociating the arena-bound and room-bound landmarks. Under those circumstances, the rats can navigate using the arena and/or room reference frame. The ability to organize the information about the two frames of reference can be impaired by relatively slight interference with the hippocampal function.

In order to study how different simultaneously relevant representations are coordinated, a task is required, in which at least two distinct representations are relevant and active. In the spatial context this can be achieved by putting a rat on a slowly-rotating arena where it is exposed to two distinct continuously dissociated sets of landmarks (Fenton et al., 1998). Under these conditions the rat's position can be defined in two spatial frames of reference. At any moment the rat's position relative to stationary room landmarks can be determined, and at the same time the rat's position relative to rotating landmarks on the arena can be defined. This is somewhat analogous to the situation of a child on a rotating carousel. The position of the child can be defined relative to the streets and buildings around the carousel, and at the same time relative to the wooden horses on the carousel itself.

On a rotating arena the rat can be reinforced to organize its behavior according to one spatial frame of reference ignoring the other (Cimadevilla et al., 2000; Fenton and Bureš, 2003), or the rat can be trained to follow both reference frames (Fenton et al., 1998). This thesis thoroughly characterizes the rat's behavior on the rotating arena when both spatial frames are reinforced (Kelemen and Fenton, 2008). Hippocampal place cells were previously recorded from rats on a rotating arena and firing fields were observed in one or the other or in both frames of reference (Zinyuk et al., 2000; Kelemen and Fenton, 2008).

Experiments of Cimadevilla et al. (2001) and Wesierska et al. (2005) suggest that hippocampus not only represents the two frames of reference but is directly involved in coordinating them. When one hippocampus was inactivated by TTX the rats could not

solve a task that required coordination of the two spatial frames. The rats were not able to learn to avoid a room-defined sector while the rotating arena landmarks were available but irrelevant. The conflict between the two frames could be removed either by stopping the arena rotation, by turning off the lights and therefore eliminating room landmarks, or by putting shallow water on the arena and eliminating landmarks on the arena surface. If any of these manipulations was used to decrease the demand for coordinating two spatial frames then the rats with unilateral hippocampal inactivation were able to perform the task. This suggests that the unilateral TTX inactivation interfered with the ability to coordinate two spatial frames rather than with spatial aspects of the task per se.

Kao and Fenton (2007) showed that injection of PCP into dorsal hippocampi impairs place avoidance on a rotating arena when room frame and arena frame of reference are both available, but only room frame information is relevant for the task. When landmarks on the arena were covered by shallow water, so that the conflict between stable room landmarks and rotating arena landmarks was diminished, the rats with hippocampal PCP injection had normal place avoidance. This result also supports the idea that hippocampus is involved in organizing distinct spatial frames.

Olypher et al. (2006) investigated hippocampal single unit activity after unilateral hippocampal inactivation. In urethane-anesthetized rats they recorded from the uninjected CA1 after TTX injection into the contralateral hippocampus. They observed increased correlation of discharge between neurons that did not have correlated activity before TTX injection. Their results suggest that coordination of discharge between distinct neurons may play a role in coordinating distinct representations. Kao and Fenton (2007) reported similar results. They showed a relationship between the impairment in coordinating two spatial frames and increased correlation in hippocampal discharge.

The goal of this thesis is to characterize neuronal mechanisms underlying coordination of two distinct representations. An experimental paradigm, in which arena and room spatial reference frames are dissociated by arena rotation is suitable for our project. We used a version of the two-frame task in which the rat was reinforced to avoid two shock zones – one defined in the room frame, and the other in the arena frame. We call the task room+arena+ place avoidance task and it is described in more detail in chapter 2.1. The hippocampal single unit activity was studied for two reasons. The well-

characterized spatial responses of hippocampal units suggest that the two spatial reference frames may be processed in the hippocampus. Furthermore, the experiments from our lab show that the hippocampus is not only involved in the processing of spatial information, but may be directly involved in coordinating the two representations.

Specific aims

Our general aim is to study how distinct, simultaneously relevant spatial frames are coordinated in hippocampal discharge. The specific aims are organized around three hypotheses of possible coordination of multiple representations.

The first aim is to study whether neurons recorded within the same session code for the two spatial frames independently, or whether cells that process the same frame are more likely to be recorded together. The independent feature detector hypothesis asserts that different cells code independently for different features of the environment. This theory predicts that different simultaneously recorded cells could be independently assigned to one or the other spatial frame of reference. On the other hand the population-coding hypothesis of hippocampus as an attractor network predicts that simultaneously recorded cells should be controlled by the same set of landmarks.

The second specific aim is to study whether different representations are organized in time on the scale of the theta rhythm. The temporal coordination hypothesis predicts that neurons coding for the same representation are synchronized, while neurons coding for different representations are desynchronized. We will study characteristic synchrony in firing of hippocampal cells, and its potential relationship to the representations the neurons code for.

The third specific aim is to determine whether the hippocampal representation is stationary throughout the experimental session or whether there is an indication of switching between different network states. One way to accommodate multiple representations in the hippocampal activity within a single session is to activate them at different times. According to this hypothesis the hippocampal representation could be switching between two different network states. We will look for evidence of such spontaneous switching between different states in ensembles of simultaneously recorded cells.

2.1. Place avoidance task

We chose the room+arena+ place avoidance task to study neural mechanisms underlying the ability to coordinate multiple spatial representations. In this task the rat is on a continuously rotating arena, and two distinct spatial reference frames are present (Bureš et al., 1997, Fenton et al., 1998). The landmarks on the arena define the arena frame of reference, and the landmarks of the room define the room frame of reference. The rats avoid an arena shock zone and a room shock zone, which are defined in the arena frame and the room frame, respectively. We can therefore infer presence of two spatial representations that are active in combination to achieve the avoidance behavior.

Room+arena+ place avoidance task

The rat was placed on a metal disk-shaped arena (82 cm in diameter, 76 cm above the floor). A transparent Plexiglas wall (40.5 cm high) was attached to the periphery of the arena, preventing the rat from jumping away. The arena was situated in an experimental room and was surrounded by a black circular curtain. The arena was continuously rotating at the slow speed of one revolution per minute. At each instant, the rat's position could be defined relative to the reference frame of the room, and relative to the reference frame of the arena. The rat's position in the room could be recognized by a large white cardboard sheet hanging on the curtain, an opening in the curtain that allowed the experimenter access to the arena, as well as by other visual and auditory cues. The position in the arena could be recognized by tactile and olfactory landmarks left by the rat on the arena surface, as well as by visual landmarks (stripes of tape) on the Plexiglas wall of the arena. The arena surface was not cleaned thoroughly between experimental sessions; only feces were removed, leaving more arena-landmarks for the rats to use.

The rat was reinforced to avoid two shock zones, the room shock zone was defined relative to the room landmarks, and the arena shock zone was defined relative to the arena landmarks. The shock zones spanned 45º and the outer 60% of the arena radius (Fig. 1A,B). When the rat entered a shock zone a mild foot shock (0.3 mA) was delivered for 500 ms. Because both the arena landmarks and the room landmarks served as conditioned stimuli, this task was called room+arena+ place avoidance. In order to

reinforce the rats to walk on the arena, they were food deprived to 85% of their free-feeding weight and trained to forage for food pellets that where scattered at random locations of the arena every 20 seconds from an overhead feeder.

The position of the rat and the displacement of the arena within the room were monitored by two light emitting diodes (LEDs), one LED was attached to the rat and the other to the arena periphery. During training prior to electrode implantation, the rat's LED was attached to the back of the rat's neck. After the electrode implantation, the LED was connected to the buffer-amplifiers on the rat's head. (The next chapter provides more details on the recording.) The arena LED was placed on a 19 cm long stick on the arena periphery 14 cm below the disk surface. The position of the two LEDs was tracked by an overhead camera and processed by a computer in the adjacent room. iTrack software (BioSignal Group, NY) was used to track the rat's position in the room and on the arena, to control the delivery of shocks and food pellets, and to store the behavioral data for further analysis.

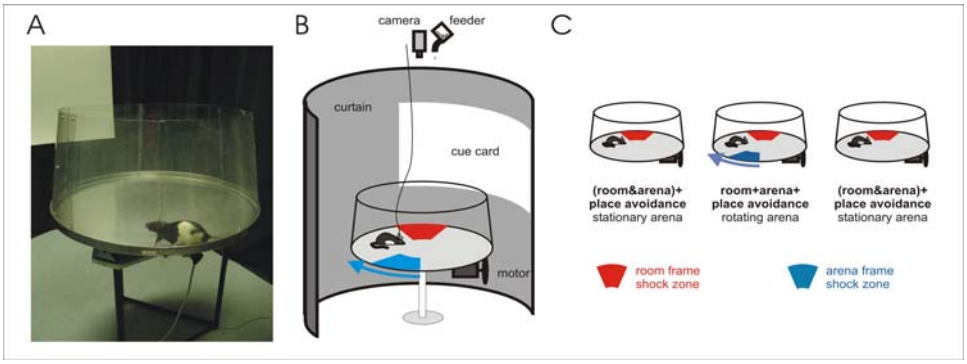

Figure 1. A photograph of a rat on an arena used for room+arena+ place avoidance task. Black curtain and a white cue card are visible in the background.
B) Schematic drawing of the room+arena+ place avoidance task. A rat was placed on a slowly rotating (1rpm) circular arena screened by a black curtain with a white cue card. The rat was food deprived and reinforced to walk on the arena and collect food pellets that were periodically (every 20 sec) scattered at random locations on the arena surface from an overhead feeder. The rat was also reinforced to avoid two shock zones. The room frame shock zone (red) was defined relative to room landmarks and did not rotate. The arena frame shock zone (blue) was defined relative to arena landmarks and rotated together with the arena as is shown by the blue arrow. The position of the rat and angular displacement of the arena in the room was monitored from an overhead camera. The cable connected to the rat's back was used to deliver foot-shocks, it also supplied power for the LED on the rat used to track the rat's position. When electrophysiological recordings were made the cable was also used to transmit electrophysiological signals from electrodes implanted in the rat's hippocampus.
C) Experimental protocol. Ensembles of place cells were typically recorded during one session of room+arena+ place avoidance task on the rotating arena flanked by two sessions of (room&arena)+ place avoidance on the stationary arena. During the stationary sessions the arena was positioned in such a way that the room shock zone and arena shock zone overlapped.

(Room&arena)+ place avoidance task

The (room&arena)+ place avoidance task was in many respects similar to the room+ arena+ place avoidance, but the arena was not rotating. Spatial information provided by the room and the arena landmarks was consistent. In the (room&arena)+ place avoidance task the rat was placed on the same circular arena, it was reinforced to avoid the same shock zone, and it was retrieving pellets scattered at random locations on the arena surface. In our experiments the same rats were trained for room+arena+ place avoidance on the rotating arena and for (room&arena)+ place avoidance on the stationary arena. During the two conditions the position of the shock zones relative to room and arena landmarks was kept constant.

Behavioral training

The rats were first food deprived to 85% of their free-feeding weight. Then they were trained for the room+arena+ place avoidance using a shaping procedure of three phases. In the first phase of shaping, the rat was trained to forage for food pellets randomly scattered on the arena. The arena was stationary (not rotating) during this phase and the rat was not receiving foot shocks. The first phase of training lasted until the rat was comfortable walking on the arena (which usually took two to five 20-minute sessions). During the second phase of training, the rat was trained for (room&arena)+ place avoidance. It was collecting food pellets on the stationary arena, and was reinforced to avoid a shock zone. This phase of training lasted until the rat was familiar with the task (again, usually two to five sessions). During the third and the last stage of training, the rat was presented with the room+arena+ place avoidance task. During this stage, one session of room&arena+ task on a stationary arena was followed by a session of room+arena+ place avoidance on a rotating arena. The rat was collecting food pellets and was reinforced to avoid both room shock zone and arena shock zone. The rats were exposed to one or two training sessions per day.

Experimental protocol

After training for the room+arena+ place avoidance task the rats were implanted with movable electrodes under pentobarbital anesthesia. For one week after the surgery

the rat was left to recover in their home cages and observed for signs of surgery-related complications. Then the electrodes were gradually lowered in search for hippocampal neurons. During this period the rat was still trained for the room+arena+ place avoidance. When electrode tips reached the hippocampal CA1 pyramidal layer and ensembles of distinct cells were detected, the recordings were made during room+arena+ place avoidance task on rotating arena and during (room&arena)+ place avoidance on stationary arena. The same ensemble of cells was recorded on the same day during room+arena+ place avoidance (15-25 min session duration) flanked between sessions of room&arena+ place avoidance (15 min duration) (Fig 1C).

Intrahippocampal injections

Five rats were trained until performance was asymptotic in the place avoidance tasks with the arena stable (10 min) and rotating (15 min) before undergoing surgery to implant a pair of guide cannulae with the tip 3 mm above the injection target in the dorsal hippocampus (relative to Bregma AP 3.5 mm; L 2.5 mm; DV 4 mm). The detailed procedure for implanting and subsequently injecting the rats has been published (Klement et al., 2005; Wesierska et al., 2005). A week after surgery the rats were retrained to re-establish optimal place avoidance performance. One rat could not regain the pre-surgical level of performance and was excluded from further study. Before testing the effect of the TTX injection on place avoidance, the rats received a bilateral injection of TTX (5 ng/µl/side) and were left in the home cage to habituate them to the procedure.

A within-subjects design was used to test the effect of injecting TTX into one hippocampus. Each rat was injected and allowed to rest in the home cage until training in the familiar task began one hour later. The injection infused either saline or TTX into the left or right hippocampus. Performance after injections was compared to sessions in which no injection was given. Each rat received the four possible injections and the un-injected session in a counterbalanced order. There was an interval at least one day between injections.

2.2. Single cell recording

Tetrodes were used to record single action potentials from pyramidal cells and interneurons of the CA1 region of the dorsal hippocampus.

Implant

Recording electrodes were made of nichrome wire 25 μm in diameter. Four wires were twisted together into a bundle called a tetrode (McNaughton et al., 1983; Recce and O'Keefe, 1989; O'Keefe and Recce, 1993). The closely adjacent tips of the four electrodes of a tetrode allowed recording of activity of the same cells on multiple electrodes simultaneously. This provided an advantage when spikes emitted by different cells were separated. Although spikes of two different cells might appear very similar on some of the four electrodes they were likely to be distinct on at least one of the electrodes (Fig. 1A).

Eight tetrodes were built into a custom made implant designed by Bruno Rivard and Robert Muller (Rivard 2002, Fig. 1B). The implant serves two functions. It provides mechanical and electrical connections between the electrodes and the recording electronics. It also provides a way to move the chronically implanted tetrodes within the brain. Each of the tetrodes is attached to a screw and can be independently advanced by turning the screw. This allows the experimenter to implant electrodes above the CA1 pyramidal cell layer, and then gradually lower the electrodes close to the pyramidal cell bodies to record electrophysiological signals from them.

Surgery

Before the surgery, the tips of the electrode wires were cut with sharp scissors, cleaned by bubbles, and gold-plated so that the resulting impedance was between 50–200 kΩ (Fig. 2). The surgery was performed under pentobarbital anesthesia (Nembutal, 50 mg/kg). The skull was exposed, cleaned and five holes were drilled into the skull. Bone screws were placed into the holes to provide mechanical support for the implant. The electrodes were stereotactically placed above the dorsal hippocampus (3.8 mm posterior, 2.5 mm lateral to Bregma, 1.5 mm below the brain surface, according to Paxinos and

Watson (1986). The implant was attached to the skull and the bone screws by Grip cement (Dentsply, Milford, DE). A ground electrode – nichrome wire 75 μm in diameter was soldered to one of the screws on the skull.

Screening for units, recording

In order to place electrodes close to the cells in the pyramidal layer of CA1 the tetrodes were moved down in steps of 30–60 μm. After the tetrodes were advanced, the experimenter waited for at least six hours for the tissue to settle, and then the electrophysiological signal from the electrodes was investigated. If the action potentials from putative hippocampal pyramidal cells were not detected, or their amplitude was not high enough to separate them from the background activity, the electrodes were advanced further.

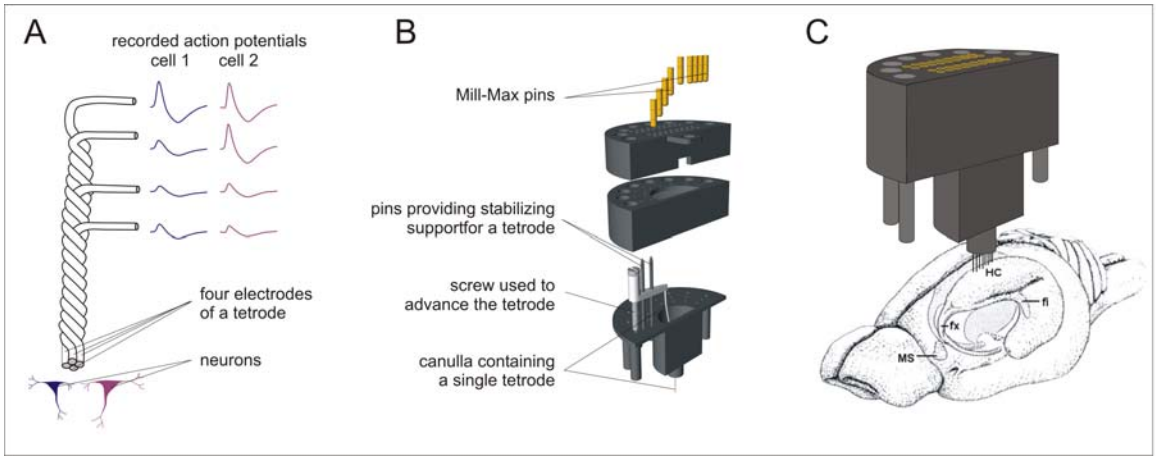

Figure 1. Tetrode recording. A) A tetrode consists of four 25 micron nichrome electrodes twisted together. Tips of electrodes are so close together that they record action potentials emitted by the same cells. The figure illustrates a situation when two cells (blue and purple) are simultaneously recorded. The average shapes of action potentials from all four electrodes are shown. Note that the action potentials from the two different cells recorded from the electrode on the top look very similar. If only the signal from this electrode was available the two cells probably could not be discriminated. At the second electrode from the top the signal from the two cells looks very different. This electrode therefore allows a discrimination of the two cells.
B) A schematic drawing of an implant. On the very top are Mill-Max pins to which electrode wires (not shown) are connected. Four of the wires are twisted together to form a tetrode and each tetrode is threaded through a canula. The canula is attached to a driving screw by a plastic holder (white). The canula can be advanced deeper into the brain by turning the screw. For simplicity the mechanism of attachment is shown only for one tetrode, in practice eight canulas, eight driving screws, and eight tetrodes are placed in a semicircle within a single implant. (Drawing courtesy of Bruno Rivard and Jeremy Barry)
C) Drawing of an assembled implant showing its position relative to the rat brain, and the dorsal hippocampus.

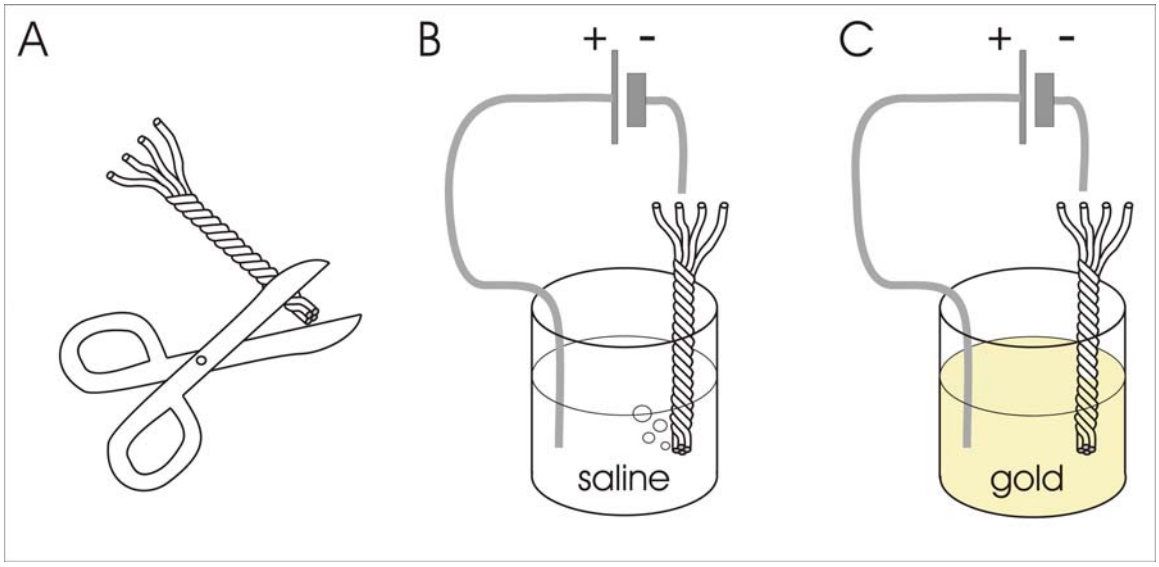

Figure 2. Preparation of electrodes prior to implantation. A) Electrodes were cut with sharp scissors. B) "Bubble test" – the tips of electrodes were cleaned by applying voltage of 5V for 20-30 seconds. C) The tips of electrodes were submerged into gold-plating solution (Sifco, Independence, OH) and gold plated by applying three to five pulses of +1V for 400 ms. The resulting impedance of electrodes was 50 - 200 kΩ.

The high impedance signal from the electrodes was sent into the source-follower pre-amplifier on the rat's head. From there a low impedance signal was passed through a recording cable to A-M Systems amplifiers and amplified 10,000 times. For single unit recording the signal was filtered between 300 and 5,000 Hz and digitized at 32 kHz. The signal for local field potential recording was filtered between 1 and 500 Hz, and sampled at 2,000 Hz.

A custom built AcX recording system and software was used to record the electrophysiological data. The software stored two milliseconds of single unit data whenever a voltage threshold was crossed. When a threshold was crossed on any one of the electrodes of a tetrode, the signal from all four electrodes of that tetrode was recorded. The EEG signal was recorded continuously together with the single unit recording.

For some of the recordings an Axona digital recording system was used (Axona Ltd, St. Albans, UK). The signal for single units was filtered between 360 Hz – 7 kHz and stored at 48 kHz. The EEG signal was low pass filtered at 500 Hz and stored at 2000Hz.

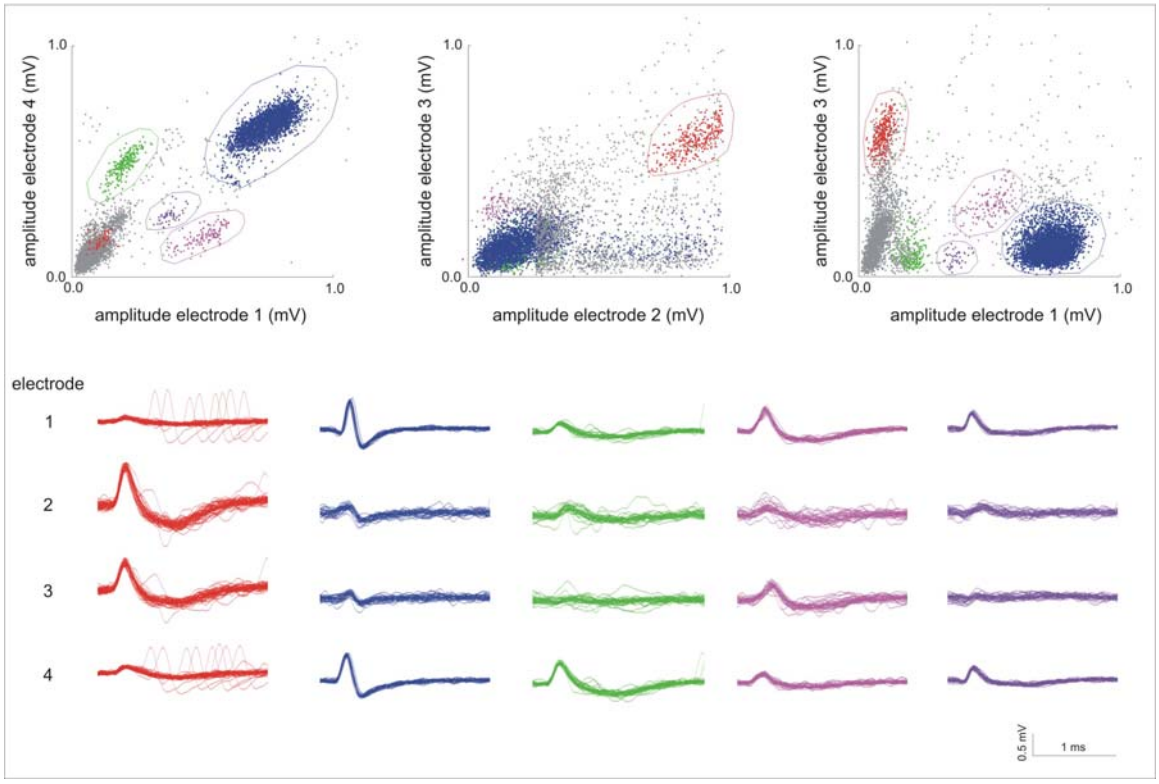

Figure 3. Separating single units from a tetrode recording. In the plots on the top each recorded event (putative action potential) is plotted as a dot according to its amplitude in one of the four electrodes of a tetrode. Notice that the dots were organized into clusters. These clusters indicate groups of action potentials with similar shape. The action potentials coming from a single neuron are likely to have similar shape and therefore each one of these clusters is thought to represent spikes from a single unit. The shape and spread of clusters show variability in amplitudes of the extra-cellular action potentials recorded from the same unit. In spite of this variability in action potentials belonging to the same cluster, the separation between clusters is apparent. The boundaries around clusters were drawn by hand.
Lower plot shows superimposed thirty action potentials for each of the clusters as they appeared on each of the four electrodes.
The blue cluster is bigger and denser than the other clusters indicating that the cell was firing at a higher rate. Furthermore, it has narrower action potential than the other units. This suggests that the blue cluster represents action potentials of an interneuron, the other clusters with lower firing rates and broader action potentials are putative pyramidal cells.

After the hippocampal signal was recorded the action potentials emitted by different neurons were separated using custom software (Wclust). The action potential separation was based on the assumptions that spikes generated by the same cell have similar shape, and spikes generated by different cells have different shape. Each single recorded event – a putative spike – was characterized by parameters such as the peak voltage, minimal voltage, voltage at a particular time from the spike onset and others. These parameters were available for each of the four electrodes of a tetrode. Any combination of two of

these parameters can serve as axes of a scatter plot, and each recorded event (spike) can be represented by a single point on that plot (Fig. 3). The points are often not distributed randomly but are organized into clusters of events with similar characteristics of action potentials. These clusters – thought to comprise spikes generated by a single cell – were separated by the experimenter.

2.3. Characterizing spike train of a single neuron

What can we learn about organization of a single cell's discharge using inter-spike intervals and autocorrelation?

To understand how information is processed in a neuronal network, it is necessary to know what types of neurons form the network, and how different neurons work together. Histological analysis gives many insights into the types of neurons present in a network and their connections. Electrophysiological recordings are a source of information of functional characteristics of different neurons. Based on the rate of discharge and temporal organization of neuronal firing in anesthetized or freely-moving animals one can identify different subtypes of neurons electrophysiologically (Ranck, 1973). Timing of neuronal activity relative to field potential oscillations can help to distinguish different types of neurons and to understand their function (Buzsáki and Eidelberg, 1983; Fox et al., 1986; Klausberger et al., 2003). Temporal organization of firing between simultaneously recorded neurons, determining which neurons fire together, and which fire in distinct times, provides useful clues about information processing in a network (Harris et al., 2003; Engel et al., 1991). This and the following chapters present tools that allow characterization of the firing of neurons within a network. In this chapter we present tools to characterize a time sequence of action potentials generated by a single neuron.

Figure 1 shows a *raster plot* of action potentials of a hippocampal neurons recorded while the rat was walking on an arena.

Characteristic patterns in timing of spikes of the cell can be detected using histograms of inter-spike intervals (Fig. 1A). This plot shows the distribution of intervals between two subsequent action potentials. The *inter-spike interval histogram* of the cell has a peak at five milliseconds. This suggests that the cell is likely to fire action potentials in close succession. Indeed, bursts of 2 to 5 action potentials fired 5-8 ms from each other are characteristic for so called complex-spike cells in hippocampus, and the cell on figure 1 is an example of this type of neuron.

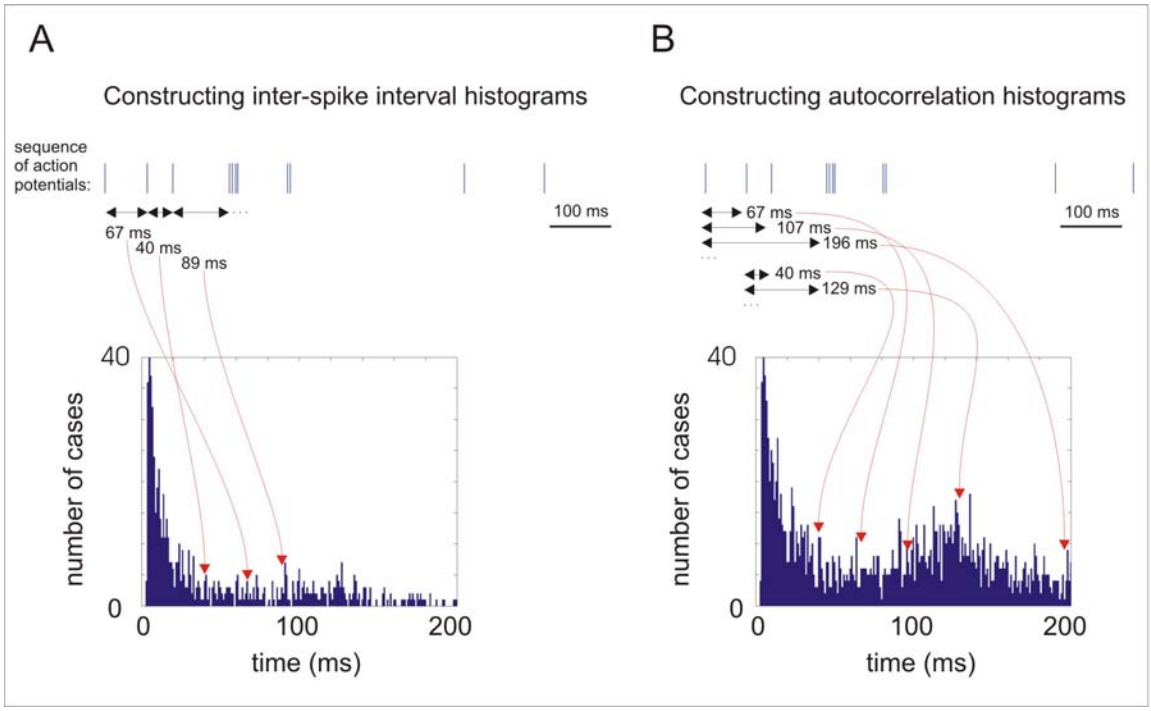

Figure 1. Constructing inter-spike interval histogram and autocorrelation histogram.
A) Inter-spike interval histogram shows distribution of intervals between subsequent spikes. On the top, a short sequence of action potentials from a single neuron is shown. Intervals between subsequent spikes at the beginning of the sequence are shown. The complete inter-spike histogram is shown on the bottom. Observed peak at 5 ms indicates the tendency of this neuron to fire action potentials in so-called complex spikes.
B) Autocorrelation plot shows distribution of intervals between all pairs of spikes. The same short sequence of action potentials as in A is shown again. Bellow, intervals between some pairs of action potentials are shown. Autocorrelation plot is shown at the bottom. Peak at 5 ms is observed again, and a bump at approximately 120 ms is present. This bump corresponds to the phase of theta rhythm, and suggests that the cell's firing was modulated by theta.

Autocorrelation histogram (Fig. 1B) is very similar to the inter-spike interval histogram with one important difference; while only intervals between pairs of subsequent spikes are included in inter-spike histogram plot, the autocorrelation plot shows intervals between all possible pairs of spikes (Rieke et al., 1997).

To create an auto-correlogram, we start with the first spike of the sequence. If the second spike occurred 67 ms after the first one, we add one count to the bin of the auto-correlation plot corresponding to 67 ms. If the third spike occurred 107 ms after the first one; we add one count to the bin 107 of the plot. We continue like this until the end of the time sequence always adding counts to bins corresponding to the time between the first and any other spike. Then we start with spike number two, and compute times between the second and all the other spikes, adding counts to the corresponding bins of the autocorrelation plot. This way we include intervals between all possible pairs of spikes.

Autocorrelation plot in figure 1B shows a tendency of the cell to fire within short intervals, corresponding to complex spikes. This we already saw in the inter-spike interval histogram. We may also notice a peak at a time of approximately 120 ms. This indicates that there is a rhythmic modulation of firing of the cell with frequency of approximately 8 Hz. This modulation corresponds to the frequency of the hippocampal theta rhythm.

2.4. Correlation of activity of a pair of neurons

In the previous chapter (2.3.) we characterized the temporal organization of discharge of a single neuron, now we will introduce methods to characterize the coordination of activity among several simultaneously recorded neurons. In this chapter we will discuss the simplest case – a correlation of firing between a pair of neurons.

In chapter 1.2. we mentioned theoretical reasons for temporal coordination of activity between different cells. For example, temporal coordination at timescale of milliseconds or tens of milliseconds was suggested as a solution to the binding problem. It has been hypothesized that the firing of two cells participating in the same representation should be correlated positively while firing of cells coding for different representations should be uncorrelated or correlated negatively (von der Malsburg, 1999). The discharge of neurons can be organized at different timescales. Attention-like phenomena operate at a timescale of hundreds of milliseconds to seconds (Donahue and Griffitts, 1931). Modulation of hippocampal discharge at this timescale has been predicted by Olypher et al. (2002), based on results of Fenton and Muller (1998). In the neocortex and hippocampus, the modulation of single unit activity on the timescale of hippocampal theta (4-12Hz) and gamma (40-100Hz) rhythms was reported (Fox et al., 1986; Engel et al., 1991; Csicsvari et al., 1999). The organization of neuronal activity at this timescale may be significant for synaptic plasticity (Larson et al., 1986; Pavlides et al., 1988; Otto et al., 1991; Hölscher et al., 1997; Hyman et al., 2003) as well as for organizing different representations (Engel et al., 1991, Harris et al., 2003).

To assess the organization of discharge on different timescales may require different analytical approaches. We will use four methods to analyze the correlation of activity between pairs of hippocampal neurons: Pearson's correlation coefficient (r), Kendall's correlation coefficient (τ), cross-correlation, and spatial-temporal covariance.

Pearson's correlation (product moment correlation)

Figure 1B shows the number of spikes emitted by two simultaneously recorded place cells with similar spatial activity during different passes through their firing fields

(Fig. 1A). The number of spikes was counted for five second intervals when the expected firing rate of each of the two cells was at least 1 spike/sec. From figure 1B one can see that when one neuron fires a lot the other neuron also fires a lot and vice-versa, the activity of the two cells is positively correlated.

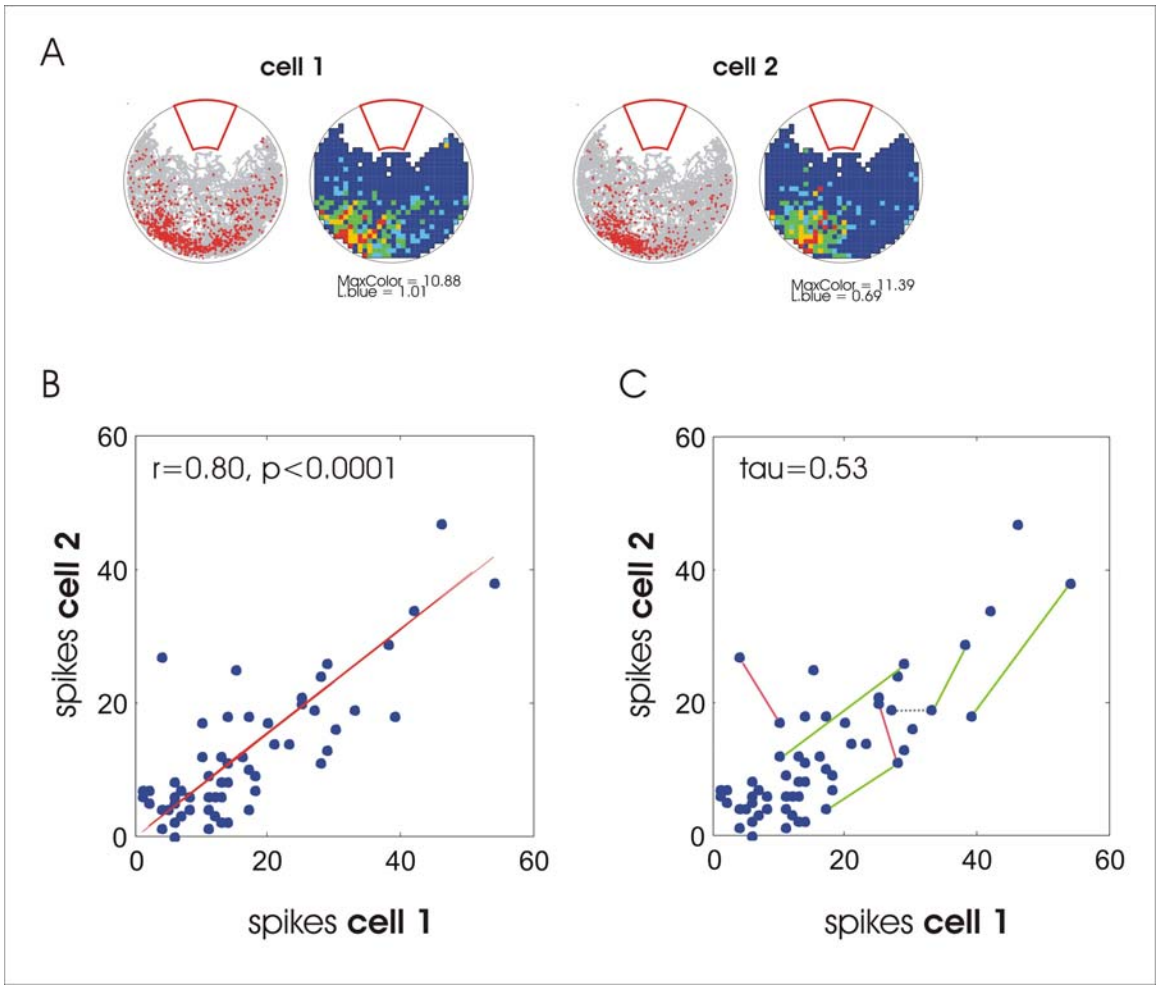

Figure 1. Analyzing the temporal relationship between discharge of two neurons.
A) Spike maps and firing rate maps of two simultaneously recorded place cells with overlapping firing fields recorded from different tetrodes. On spike maps the rat's trajectory during a recording session is shown in gray and locations where the cell discharged action potentials are shown in red. The activity of these two place cells is analyzed in parts B and C of this figure, and in figure 4.
B) The firing of the two place cells was characterized during those 5-second intervals when the rat was crossing firing fields of both cells (both cells were expected to fire at least 5 spikes). We can see that the number of spikes emitted during different passes through the firing fields are correlated. Pearson's correlation coefficient ($r=0.8$; $r^2=0.64$) indicates that 64% of the variability of firing of cell 2 can be explained by firing of cell 1.
C) Kendall's τ is another way to assess the relationship between two variables. It is based on counting the number of concordant and discordant pairs of events. Lines connecting concordant pairs have positive slope, some of them are marked by green color. Lines connecting discordant pairs have negative slope and some of them are marked by red color. Event pairs that have equal X or Y value (one example marked by gray dashed line) are neither concordant nor discordant. In this case there was more concordant pairs, therefore Kendall's correlation coefficient was positive, $\tau=0.53$.

The most commonly used measure of correlation between two variables is Pearson's correlation coefficient r (also called product moment correlation), and it is computed according to the following formula:

$$r = \frac{\sum_{i=1}^{n} (X_i - M_x)(Y_i - M_y)}{(n-1)S_x S_y}$$

where X_i and Y_i are spike counts of the two cells during different five second intervals *i*, M_X and M_Y are mean spike counts of the two cells, and S_X and S_Y are standard deviations of spike counts of the two cells, *n* is the sample size (number of passes through the firing fields). As the formula shows each value (number of spikes) is subtracted from the mean number of spikes of a particular cell. For each five seconds the product of the deviations from mean for the two cells is computed. Notice that the product is positive if both cells fire more than their respective averages, or if both cells fire less than their respective averages. If one cell fires more than the average and the other less than the average the product is a negative number. In other words, if the sample falls in the upper-right quadrant or in the lower left quadrant of the figure 1B the product is positive. Sum of these products is then computed and normalized (divided by $(n-1)S_X S_Y$) so that the resulting value lies between -1 and 1. Square of Pearson's correlation coefficient (r^2) is often reported and is interpreted as the proportion of variability in one variable that can be explained by the other variable. For the data shown on figure 1B the Pearson's r is 0.8.

Kendall's correlation

Interactions between cells on short timescales pose certain challenges to the analysis. When number of spikes emitted by a cell are counted during short time intervals, the cell often does not fire – there are many "zeros", and when it fires, it fires only few spikes (1, 2, or 3). The values of the variable fall into few categories, so the variable that we are measuring is ordinal rather than continuous. Kendall's τ is a non-parametric measure that is better suited to assess correlations of ordinal variables. This measure is also less sensitive to the extreme values (outliers).

For each pair of points on a scatter plot (Fig. 1C) a difference between the firing rates of one cell is computed and difference between firing rates of the other cell is computed. When both differences have the same sign (both are negative or both are

positive), then the pair is called concordant, if the differences have different sign, the pair is called discordant. The difference between concordant and discordant pairs can be illustrated graphically: when the line connecting the two data points has positive slope the pair is concordant (green lines on fig. 1C), when the slope is negative the pair is discordant (red lines on fig. 1C).

The difference between the number of concordant *C* and discordant *D* pairs is computed and normalized according to the formula:

$$\tau = \frac{C - D}{\sqrt{n - T_x} * \sqrt{n - T_y}}$$

If there are more concordant pairs the τ is positive – variables are positively correlated, if there are more discordant pairs the τ is negative – the variables are negatively correlated. To normalize the value, so that it falls between 1 and -1, this difference (*C-D*) is divided by the maximal expected difference. This maximal difference is computed from overall number of pairs *n* and number of pairs in which either x or y values are tied T_X and T_Y respectively (Freund and Williams, 1991).

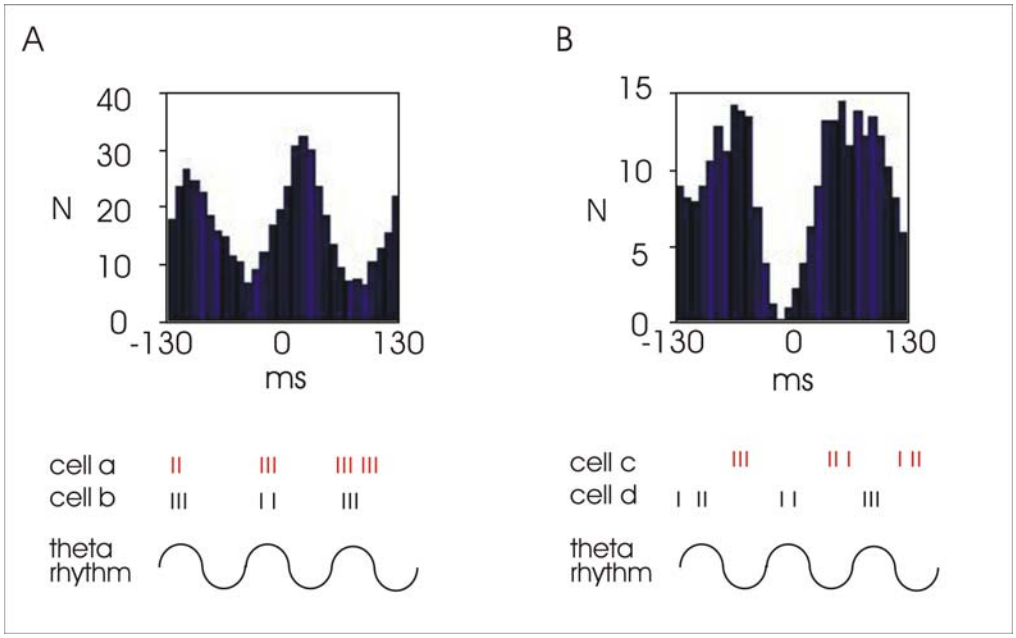

Figure 2. Cross-correlation histograms show relationship of firing of the two cells on millisecond time scale. A) For these two cells the peak of cross-correlation was close to 0 ms. This suggests that the cells tended to fire together. The other two peaks at around +130 ms and -130 ms correspond to the period of the theta rhythm. A cross-correlation like this would be expected if the two cells fired predominantly at the same phase of the theta cycle, as is shown by the cartoon below the cross-correlogram. B) The cross-correlogram shows that these two cells did not fire together, but rather approximately 70 ms apart. This could happen if the two cells discharged at the opposite phases of the theta cycle.

Cross-correlation

Another measure that characterizes the temporal relationship between pairs of neurons is the cross-correlation (Fig. 2). It is similar to the autocorrelation described in the previous chapter. A cross-correlation histogram shows the number of times the two cells fired within different time intervals. Different time intervals are shown on the abscissa, the number of occasions the two cells fired with a given time interval is shown on the ordinate. An example of cross-correlation histogram for a pair of neurons is shown in figure 2A, it has a peak close to 0 ms. This signifies that these two neurons tend to fire within a few milliseconds from each other. The other two peaks at approximately -130 and +130 ms reflect theta modulation of activity of the two cells. If both cells prefer firing at the same phase of theta rhythm, then their spikes are more likely to occur 140 ms apart than 70 ms apart. A cross-correlogram for a different pair of neurons is shown in figure 2B, it has a peak between 60-70 ms, and a valley close to 0 ms. This means that the two cells were rarely active together, and often fired 60-70ms apart.

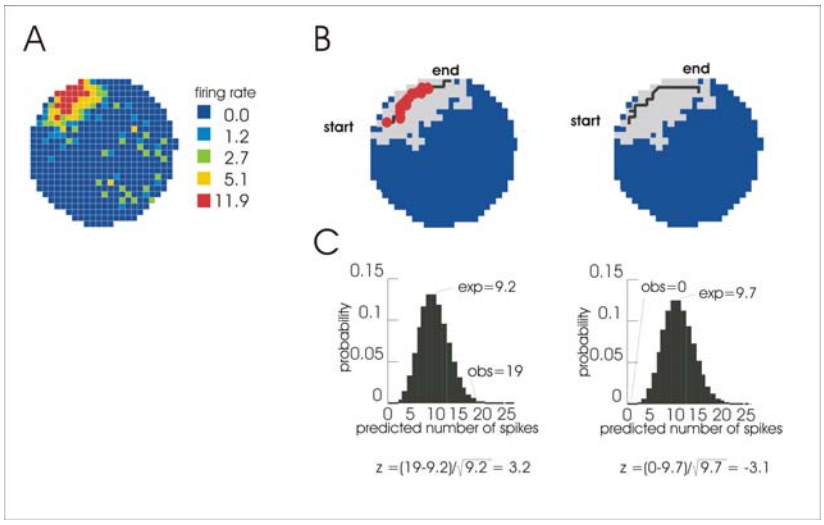

Figure 3. Excess variance in firing of a place cell and standardized firing rate.
A) Firing rate map of a place cell. Activity of this place cell is analyzed in parts B and C of this figure.
B) Two different passes through the firing field of the cell. The firing field is shown in gray, rat's trajectory in black, and action potentials are marked by red dots. During the pass shown on the left the cell discharged 19 action potentials. During the pass shown on the right the cell did not fire at all, although the rat's trajectory was similar to the one shown on the left.
C) Transformation of observed firing rate to normalized firing rate. The histograms show probability distribution of firing rate for the two passes through the firing field. Values of expected (*exp*) and observed (*obs*) number of spikes are indicated. During the segment shown to the left the standardized firing was 3.2; for the segment on the right the standardized firing was -3.1. (Adapted from Fenton and Muller, 1998)

Spatial-temporal covariance

While Pearson's and Kendall's correlation coefficients and cross-correlation are broadly used, spatial-temporal covariance as defined here was designed specifically to study variability in place cell discharge. Fenton and Muller (1998) noticed great variability in firing rate of a place cell during different passes of a rat through the cell's firing field. On one passage through the firing field a cell may fire a lot, on another passage it may not fire at all, although the trajectories of the rat may be very similar during the two passages (Fig. 3B). This phenomenon was initially called excess firing variance and subsequently a term overdispersion of place cell discharge was used. The spatial-temporal covariance was designed to assess whether this firing variance during different passes through the firing field is correlated between pairs of simultaneously recorded cells. While the previous measures characterized the actual number of spikes emitted by two cells, to compute covariance we need to know how much the observed number of spikes deviates from the expected value. In other words, we are interested in the standardized firing rate:

$$\text{standardized rate } (z) = \frac{(obs - exp)}{\sqrt{exp}}$$

$$exp = \sum r_i t_i$$

For each pass through the firing field we calculate the expected firing rate *exp* according to the formula above. We know which pixels of the firing rate map the rat visited during a given time interval, and how much time the rat spent in each pixel. Multiplying the time spent in a pixel t_i with the average firing rate for the pixel r_i, and summing the products for all the pixels visited during given five seconds gives the expected number of spikes *exp*.

We subtract observed number of spikes *obs* from the expected number of spikes *exp*, and normalize the difference by dividing it by the square root of the expected number of spikes. This value is called standardized firing rate (Fig. 3). Pearson's correlation can then be used to assess whether the standardized firing rates of the two cells correlate and this parameter we call spatial-temporal covariance (Fig. 4). For the two cells shown in figure 1 the standardized firing rates were positively correlated, the spatial-temporal covariance was 0.6. This means that not only the absolute firing rate of

the two cells is correlated, but also the deviation of observed firing rate from the expected one – the variability in firing – is correlated.

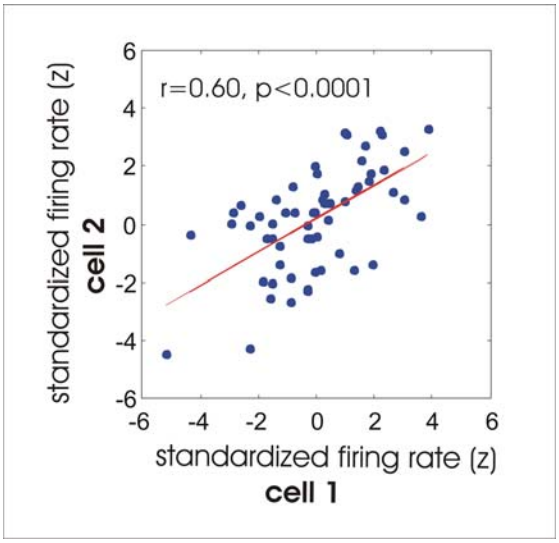

Figure 4. Spatial-temporal covariance in firing of the same pair of cells as shown in figure 1. Standardized firing rates of the two cells are compared. Pearson's correlation coefficient r=0.6 indicates that 36% of the variability of standardized firing rate of cell 2 can be explained by standardized firing rate of cell 1.

2.5. Analysis of data from ensembles of simultaneously recorded neurons.

This chapter describes tools to study activity of ensembles of multiple simultaneously recorded neurons. Ensemble vectors characterize the activity during short time-intervals, and correlations between the vectors allow comparison of activity patterns at different times. Principal component analysis allows visualizing the patterns of activity in ensembles of multiple neurons.

Spatial activity of an ensemble of simultaneously recorded place cells can be characterized by firing rate maps (Fig. 1A). The cells shown on figure 1 were recorded when a rat was performing the (room&arena)+ place avoidance task on a stable arena during two sessions in room A, and during one session in room B. In room A the circular arena was surrounded by a black circular curtain with a white rectangular sheet of paper as a cue. Similar circular arena was used in room B. Room B had a rectangular ground plan, there was no curtain around the arena, and the rat could see shelves on a wall and other furniture. Firing rate maps are similar during two different sessions recorded in the same room A, but they are different in room B. Some cells were only active in room A (e.g. cells 1 and 2), other cells were active only in room B (cells 13 and 15), and still other cells were active in both rooms, but discharged at different locations (cells 11 and 12).

The firing rate maps show beautifully the change in spatial responses of hippocampal ensemble between two environments – a phenomenon called remapping (Bostock et al., 1991). However, because the firing rate maps average activity throughout a whole recording session, they do not provide any information about potential changes in activity within a session. To study the dynamics of ensemble activity on a short timescale, one has to look at short time intervals.

Ensemble vectors

Activity of an ensemble during a short time interval can be characterized by number of spikes emitted by each of the cells. A list of spike counts of different neurons during a given time interval will be called an ensemble vector (Fig. 2A). Similarity of ensemble activity during different time intervals can be measured by computing Pearson correlation

between ensemble vectors, as is illustrated in figure 2A for two pairs of vectors. Ensemble vectors representing two one-minute intervals recorded in the same environment were highly correlated, which indicated high similarity in activity pattern. On the other hand, for one-minute intervals from different environments the ensemble vector correlation was negative, indicating distinct ensemble activity in the two environments (Fig. 2A).

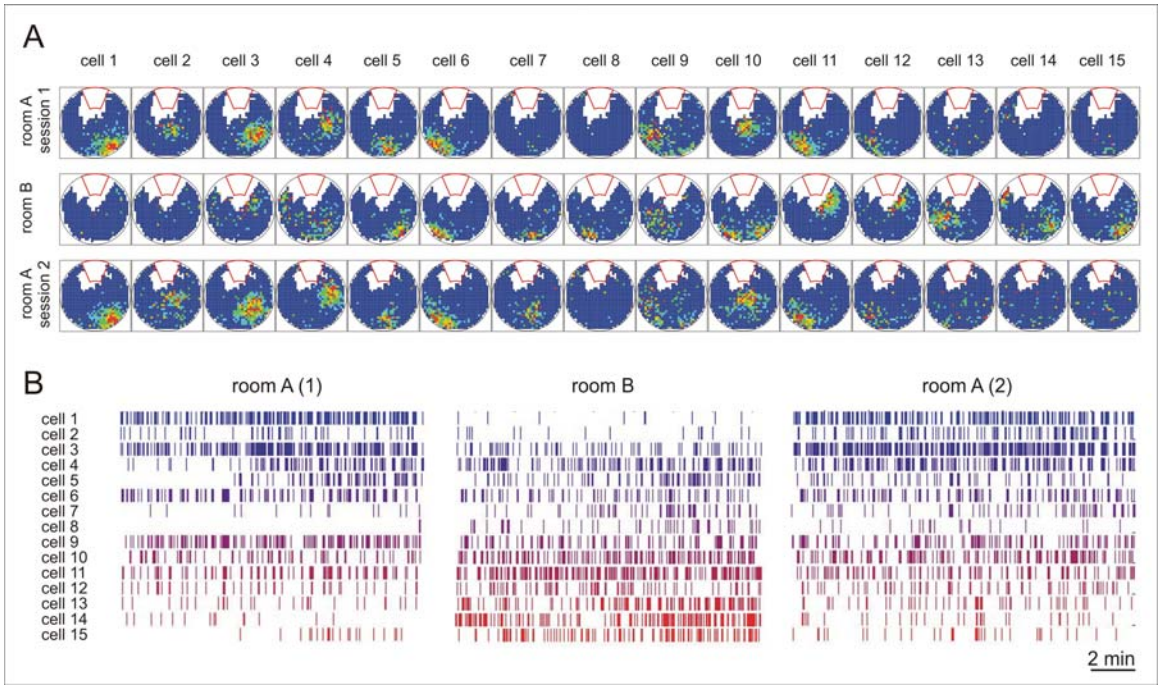

Figure 1. Activity of ensemble of 15 cells recorded in two different rooms during (room&arena)+ place avoidance task on a stable arena. A) Firing rate maps show activity of each neuron in different locations of the arena. Red color corresponds to places of maximal firing, blue indicates locations with zero firing rate. The spatial activity was similar during two sessions within the same environment, but distinct between the two environments. B) Ensemble raster plots show temporal organization of activity of each cell in the two environments.

Pattern of activity in an ensemble during a series of time intervals can be conveniently shown in the form of a correlation matrix (Fig. 2B). In the correlation matrix, each interval is compared with every other interval. The same sequence of one-minute intervals is represented on the two axes. The correlation coefficient between each pair of intervals is color-coded; red pixels correspond to positive correlation; the dark blue pixels correspond to negative correlation. The correlation matrix on figure 2B shows that ensemble vectors recorded in the same experimental room are positively correlated, indicating a consistent activity pattern throughout the recording sessions in the same

environment. Ensemble vectors from different rooms are uncorrelated or negatively correlated (Fig. 2B), indicating distinct ensemble activity in the two rooms.

Ensemble vectors and correlation matrices will be used in chapter 3.5. to characterize ensemble activity patterns during room+arena+ place avoidance on the rotating arena and to compare it to activity during (room&arena)+ place avoidance on the stationary arena.

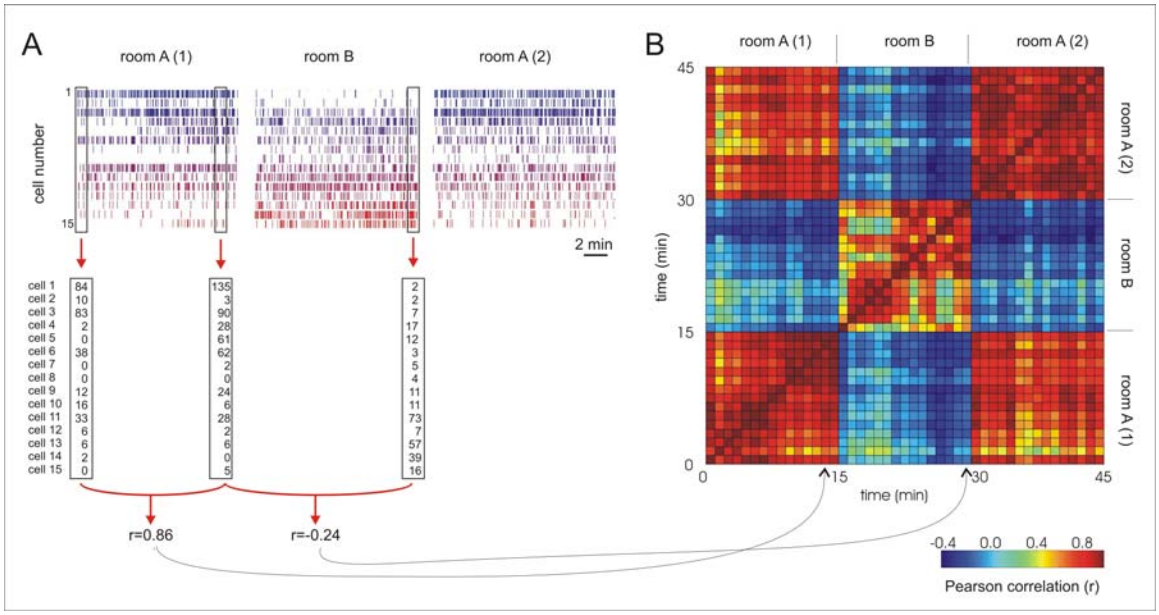

Figure 2. Ensemble vectors and their correlations. A) Ensemble activity during different one-minute time intervals can be represented by an ensemble vector. Similarity between ensemble activity during different intervals can be measured by computing the correlation between the two corresponding ensemble vectors. B) Correlations between vectors representing different time intervals are plotted in the form of a correlation matrix. Red color corresponds to high positive correlation, dark blue to negative correlation. The figure shows that during different one-minute intervals recorded in the same room the firing pattern is similar, and activity patterns in different rooms are distinct. The plot also indicates moments of decreased correlation within the same room and moments of increased correlation between the two rooms (green pixels). This may suggest changes in ensemble activity within a single room. Dynamic changes of activity pattern within a single environment will be closely studied in chapter 3.5.

Principal components analysis

When relationship between discharge of two cells is studied, it is often convenient and informative to visualize the activity of cells in a scatter plot (see chapter 2.4.), where each axis represents the activity of a single cell (Fig. 4A). With naked eye, without help of statistical tools, one can see whether there is a simple pattern in activity of the two cells, whether their activity is positively or negatively correlated, etc. It would be practical to similarly represent activity of large ensembles of cells, and look for a pattern

in their distribution. Principal component analysis can be used to simplify large multi-dimensional ensemble vectors so that they can be manipulated more practically.

The activity of three or more neurons can be represented by a point in a three or more dimensional space, firing of each neuron represented by one axis. Every ensemble vector representing activity of *n* neurons defines a point in *n*-dimensional space. If the number of neurons becomes higher than two, we can no longer fully represent the state of the system by two-dimensional drawing. Although we cannot draw three- or more dimensional space, we can project the points of high-dimensional space into a plane. This is analogous to the way painting, or photography projects three-dimensional object onto a two-dimensional canvas or screen (Fig. 3). Because not all information about a 3-D objects can be preserved in a painting, it is important to choose a projection that preserves as much information about the object as possible. (Early painters chose to draw a bull from side, rather than from behind, for example. Fig. 3)

When working with large ensembles of *n* cells we are challenged to find projection that would preserve most information about the original *n*-dimensional data. Principal components analysis (PCA) is a technique used to find this “best” projection. Principal component analysis finds a line in a multi-dimensional space with the following property; when all the points of the original data are projected onto that line, the variability will be maximal. This line is called the first principal component. The second principal component is a line with two properties; it is perpendicular to the first principal component, and when all the points of the original data are projected to the line, the variability is maximal. The first and the second principal components define a plane. If the data from the multi-dimensional space are projected to this plane the picture represents as much variability in the original data as possible. PCA generates as many principal components as there are dimensions of the original data. For each principal component PCA generates a number (eigenvalue) that indicates how much of the variation in the data is represented by each principal component.

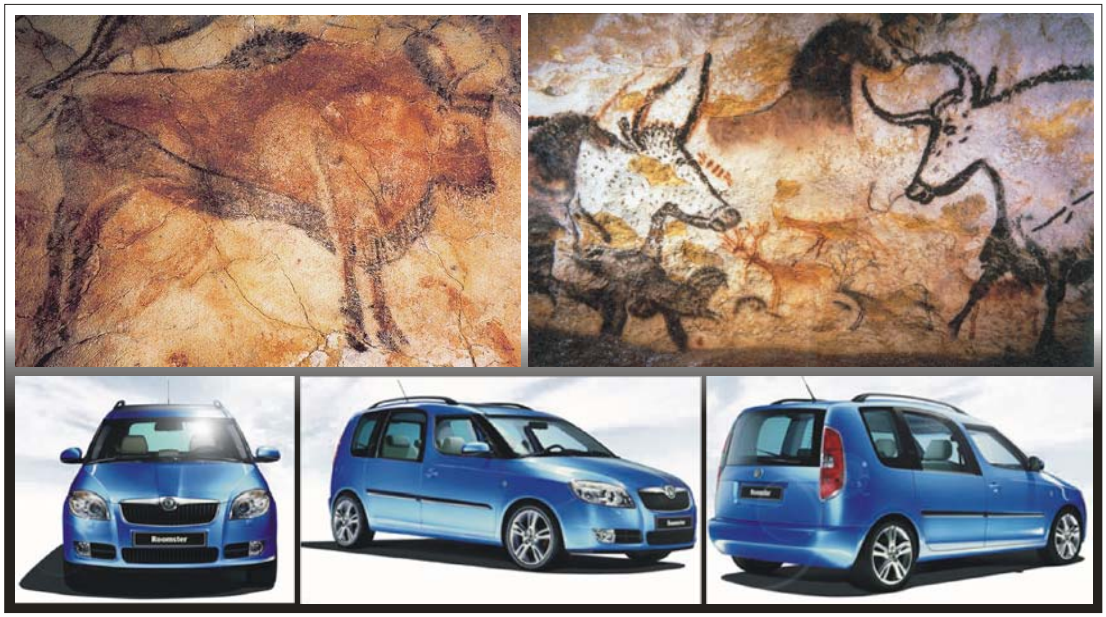

Figure 3. Showing three-dimensional objects in two-dimensional pictures is a task performed on many occasions for ages. In each case, one of infinite number of projections has to be chosen. Generally the projection that preserves the most information about the represented object is preferred.

How PCA works can be illustrated on an example of firing rates of two hypothetical neurons. Figure 4A shows number of spikes emitted by these neurons during 11 time intervals. The first principal component is shown by the red line. As was mentioned before, if all of the points are projected to this line, most of the variability is preserved. The second principal component is a line perpendicular to the first one. Figure 4B shows the same data, but now the principal components are used as axes. One can see that the points are spread along the first principal component but vary less with the second principal component. In this particular case the first principal component represents 91.5% of variance in the original data, the second principal component represents the remaining 8.5%.

The previous example used only two neurons to demonstrate how PCA works, however the real power of the PCA, that is to reduce multi-dimensional space to lower number of dimensions, was not revealed by this example. PCA can be applied to the hippocampal ensemble shown in figure 1. Again, ensemble vectors for one-minute intervals of data recorded during three sessions in two different rooms were analyzed. The ensemble vectors are shown in the coordinate system of the first two principal

components (Fig. 5). The plot reveals a structure in the data: points cluster into two groups. These two clusters represent the data recorded in the two rooms.

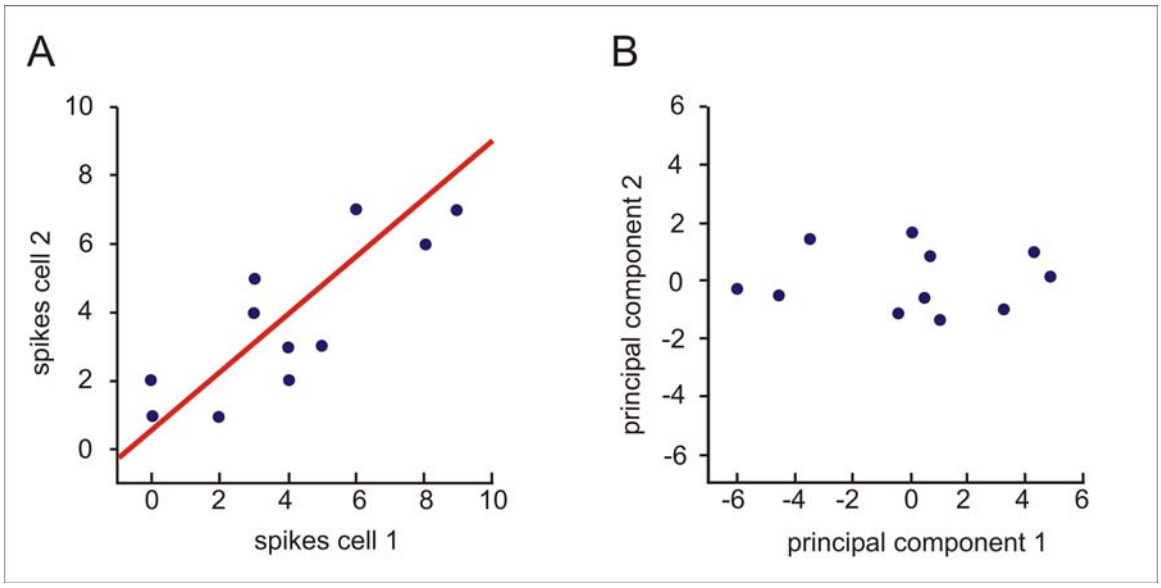

Figure 4. Left: Spike counts of two hypothetical neurons during 11 time intervals are shown on the left. Principal components of the distribution were computed, the first principal component is shown by a red line. Right: The same 11 events are represented in the coordinate system of the two principal components.

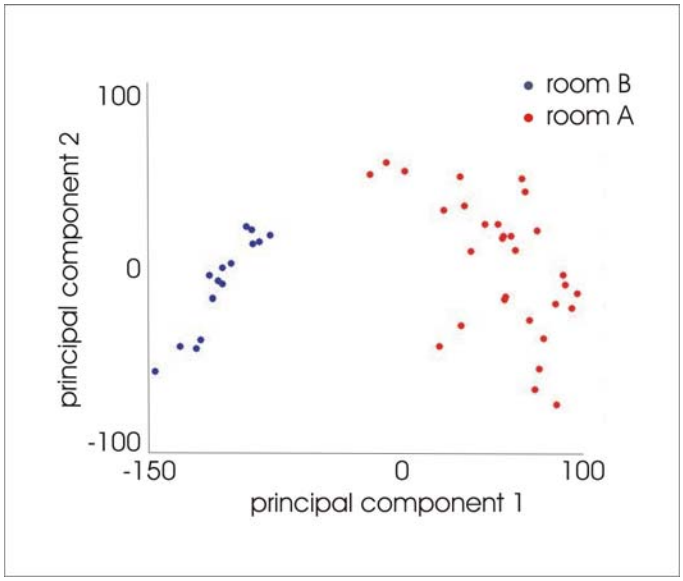

Figure 5. Activity pattern of an ensemble of neurons shown in figure 1 in two different rooms during different one-minute intervals. The ensemble vectors representing 15 neurons are shown in the projection of the first two principal components. PCA revealed difference between the activity patterns in the two rooms. The activity recorded in room A (red dots) occupies a different part of activity space than the activity recorded in room B (blue dots).

(RasterPlotsSession.m; EnsembleMatrixFromTS.m)

2.6. How to characterize spatial firing of place cells.

Parameters used to quantitatively characterize spatial organization of place cell firing are introduced in this chapter. Properties of spatial coherence, information content, and instantaneous positional information are discussed.

In this thesis we use two methods to visualize spatially organized firing of hippocampal neurons: a spike map and a firing rate map (Fig. 1A). In the case of room+arena+ place avoidance task on the rotating arena the position of the rat can be defined in two distinct spatial reference frames (room frame and arena frame). Therefore spike maps and firing rate maps can be constructed for both frames of reference (Fig. 1B). Three parameters are used to quantitatively characterize spatial organization of place cell firing. These are spatial coherence, information content, and instantaneous positional information.

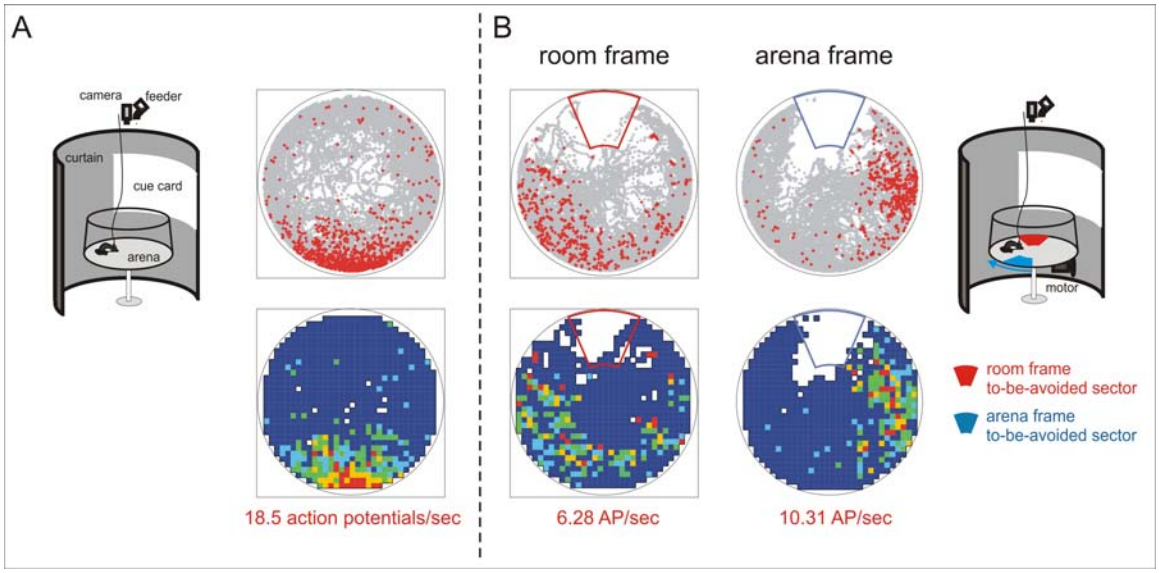

Figure 1. A) Activity of a place cell from a rat on a stationary arena. Spike map (above) shows trajectory of the rat during a recording session as a gray line and locations where the place cell discharged action potentials in red. The cell was active when the rat was in the south part of the environment. The corresponding firing rate map (below) shows firing rate in different locations of the arena. The red color marks locations with maximal firing (≥18.5 action potentials per second); the blue color marks locations with lowest activity. B) During room+arena+ place avoidance the spike maps and the firing rate maps were created for the room frame and the arena frame separately. The plots show the activity of the same cell during a single session. Schematic drawings of the recording apparatuses are shown on the sides.

Spatial coherence

Spatial coherence (Kubie et al., 1990) characterizes similarity in the firing rate between adjacent pixels of a firing rate map. Firing rate in each pixel is compared with the average firing rate in the eight adjacent pixels. The average firing rate of the eight pixels is computed by dividing the spike count in the eight pixels by the time spent in the eight pixels. Pearson correlation between the original firing rate in each pixel and the average of the eight neighbors is computed. Finally the correlation coefficient (r) is z-transformed to normalize the value for statistical comparison.

Information content

Information content was introduced by Skaggs et al. (1993) to measure the information about the rat's position in an environment provided by the cell's activity. It is computed according to the following formula:

$$I_S = \sum_{a=1}^{K} P_a \, FR_a/FR \, \log_2(FR_a/FR) \qquad (1)$$

In a modified version information per time can be computed as:

$$I_S = \sum_{a=1}^{K} P_a \, FR_a \, \log_2(FR_a/FR) \qquad (2)$$

In equations (1) and (2) P_a is probability of being in location a, FR_a is firing rate in location a, and FR is mean firing rate. Information content depends on the uniqueness of firing in different pixels of the firing rate map. Unlike the spatial coherence, information content does not reflect similarity between neighboring pixels in the firing rate map. Because spatial proximity of pixels is not taken into account during computation, even disorganized spatial activity can provide high information content. For example, the yellow cell in figure 2 has information content similar to the gray or green cell, although its firing is much less spatially organized.

Instantaneous positional information

Instantaneous positional information can be used to characterize firing during short time intervals (117 ms was used in our analysis, Olypher et al., 2002b, 2003). Instantaneous positional information $I_{pos}(t)$ during a given time interval is computed according to the following formula:

$$I_{pos}(t) = P_{i|a} \, \log_2(P_{i|a}/P_i) \qquad (3)$$

The number of spikes emitted (i), and the position of the rat (a) during the time interval (t) are used. The conditional probability of observing the emitted number of spikes (i) in current location $P_{i|a}$ is compared the probability of observing that number of spikes in the overall data set P_i. The values of $P_{i|a}$ and P_i are determined from the entire recording session. The $I_{pos}(t)$ is high if number of fired spikes (i) is more usual in the current location than overall ($P_{i|a}>P_i$). It is zero if the observed number of spikes is as likely to occur in the current location as overall, e.g. the firing rate is not particular for current location. $I_{pos}(t)$ can be positive or negative. However, the absolute value of $I_{pos}(t)$ is large whenever the number of spikes observed at the location is distinct or "surprising" compared to the overall (location-independent) probability of observing the same number of spikes. We denote $|I_{pos}(t)|$ using the shorthand, $|I_{pos}|$. The time series of $|I_{pos}|$ for a cell recorded during a session of room+arena+ place avoidance is shown in figure 3C. More examples can be found in chapter 3.2.

The properties of these three parameters are illustrated using a database of 162 putative hippocampal pyramidal cells, which were recorded in 16 sessions from 7 rats during (room&arena)+ place avoidance on the stable arena. The scatter plots on figure 2B and 2C show distribution of spatial coherence, mean instantaneous positional information and information content in the database of cells, and relationship between these parameters. The relationship between the three parameters and firing rate of cells is illustrated in figures 2D, E, and F. Figure 2A shows firing rate maps of seven of the complex spike cells. To each of the seven cells a color was assigned, this color is indicated by the rectangle around the cell's firing rate map. Colors can be used to identify the seven cells in the scatter plots of figure 2B-F.

The coherence seems to characterize the firing field the best. The cells with "better firing fields" – green, blue, gray and pink – are also cells with the highest coherence as can be seen on figure 2B. On the other hand red and yellow cells have "bad firing fields" and also the lowest coherence. Later in this work coherence of 0.4 or higher will be used as a criterion for a cell with very good firing field. The information content (IC) seems to be the least accurate parameter of the three. Although the blue cell has the firing field

organized much better than the red cell, the red cell has higher IC. Similarly, although the green cell has better firing field, the yellow cell has higher IC. The average instantaneous positional information seems to be a useful parameter to characterize spatial firing; its main advantage is in ability to characterize firing during short time intervals, as is shown in chapter 3.2.

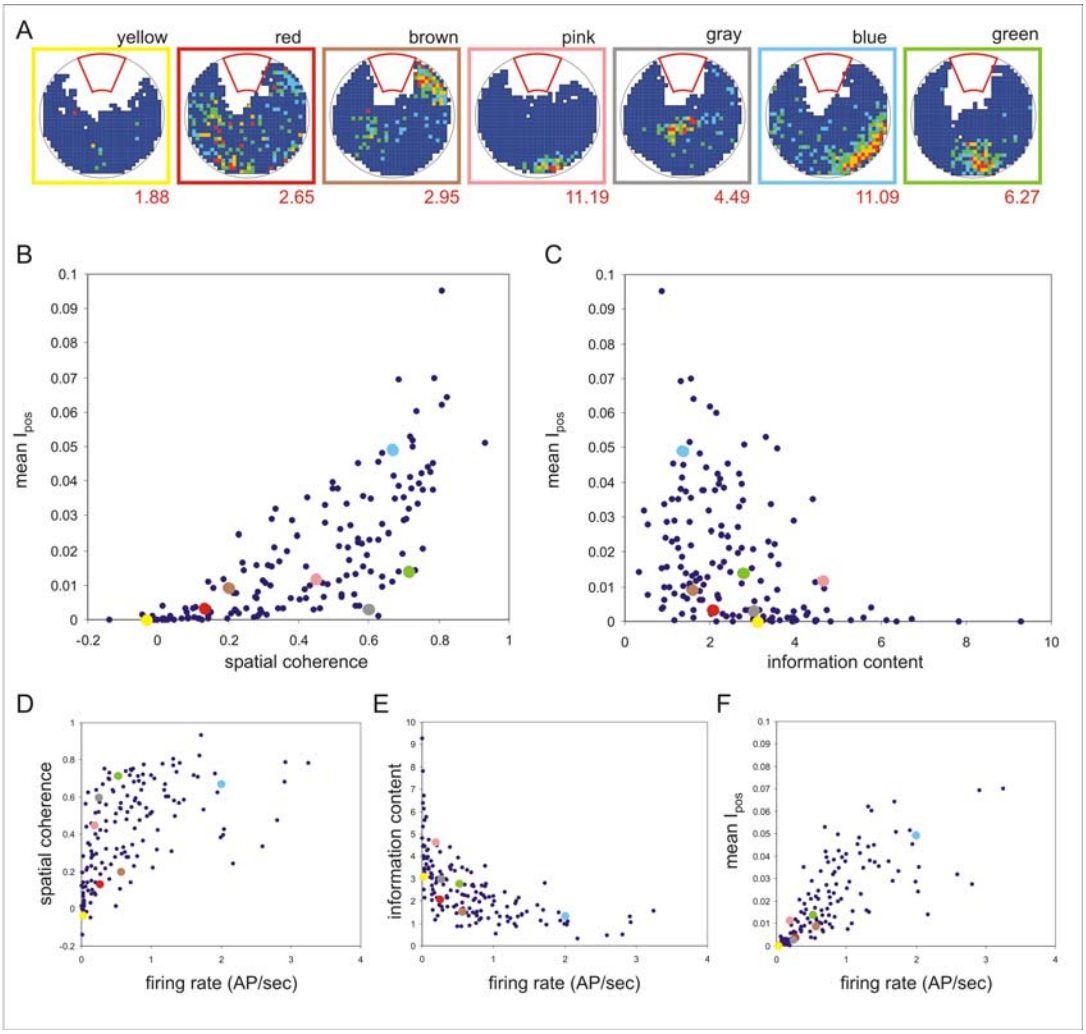

Figure 2. Three parameters used to quantify spatially selective firing of hippocampal complex spike cells. A) Firing rate maps of seven neurons, these neurons were chosen so that cells with spatially disorganized firing as well as cells with nicely organized firing fields are represented. The red number bellow each firing rate map shows the firing rate threshold (action potentials/ second) for the red pixels with the maximal firing. The scatter plots in B) and C) show coherence, mean instantaneous positional information ($|I_{pos}|$), and information content of all recorded cells. (Each dot represents a single cell.) The dots representing the neurons shown in A) are little bigger and are marked by colors corresponding to colors of the squares around firing rate maps. The plots suggest that coherence and mean $|I_{pos}|$ better reflect the subjective evaluation of firing field quality than values of information content. These two parameters will be predominantly used in the further analysis. D,E,F: Relationship between parameters characterizing spatial firing and firing rates of cells. The figure shows that spatial coherence (D) and mean $|I_{pos}|$ (F) are positively correlated with the firing rate, while information content (E) is negatively correlated with firing rate.

Figures 2D, E, and F show how the three parameters are related to the firing rate of a cell. Spatial information content (IC) tends to be higher for cells with lower firing rate. The IC reaches high values if there are pixels with firing rate much higher than average rate. Therefore it tends to be high if the rate map has only few active pixels with high rate but otherwise the cell is not active keeping the overall firing rate low. Mean $|I_{pos}|$ increases with the firing rate. Time series of $|I_{pos}|$ as function of time shows that $|I_{pos}|$ often increases at the moments of the cell's firing (Fig. 2 in chapter 3.2.), therefore the more the cell fires, the higher is the mean $|I_{pos}|$.

Organization of place cell firing in the room+arena+ place avoidance task

In the room+arena+ place avoidance task the position of the rat can be defined in the room frame and the arena frame. The spike maps and firing rate maps can be constructed for both frames of reference (Fig. 1B), and the firing in both frames can be evaluated using the parameters explained above.

Paradoxically, presence of firing fields in both reference frames is not sufficient to prove that both spatial frames modulate activity of a particular cell. In chapter 3.1. we show that locations visited by the rats strongly depend on the position of the arena in the room. Unequal sampling of different locations during different arena displacement may have a consequence of creating "fake" firing fields in one of the reference frames. For these reasons firing rate maps alone are inadequate to determine whether a particular cell has firing modulated by both reference frames, and other methods of analysis must be used. Our approach will be illustrated on an example of a cell in figure 3 with firing fields in both frames.

Figure 3 shows a cell, which discharged predominantly when the arena was displaced by approximately 210˚ relative to the room. In this configuration the location 60˚ clockwise from the room shock zone corresponded to the location 150˚ counter-clockwise from the arena shock zone. This pattern of firing would be expected if the cell's activity was controlled by both arena and room frames of reference. Before accepting that the firing was modulated by arena displacement, we tested whether the spatial activity in one frame of reference could be explained by the activity in the other frame. We created a firing rate map in the room frame that would be expected if the cell's

spatial activity was only organized in the arena frame, without any modulation by the rat's position in the room. We also created a complementary expected arena map based on room frame spatial firing (Fig. 3B). Comparison of these expected maps and their observed counterparts suggests that the spatial firing in the room frame could be largely explained by activity in the arena frame (Fig. 3B). This can be demonstrated by high correlation between the expected and observed spike distribution in the room frame (r=0.79). Dominant influence of the arena frame on this cell's firing was confirmed by analysis of $|I_{pos}|$. According to this analysis the cell's firing provided information about the arena frame position, but not the room frame position (Fig. 3C). Based on these analyses we conclude that the cell's spatial activity was predominantly related to the rat's position in the arena frame.

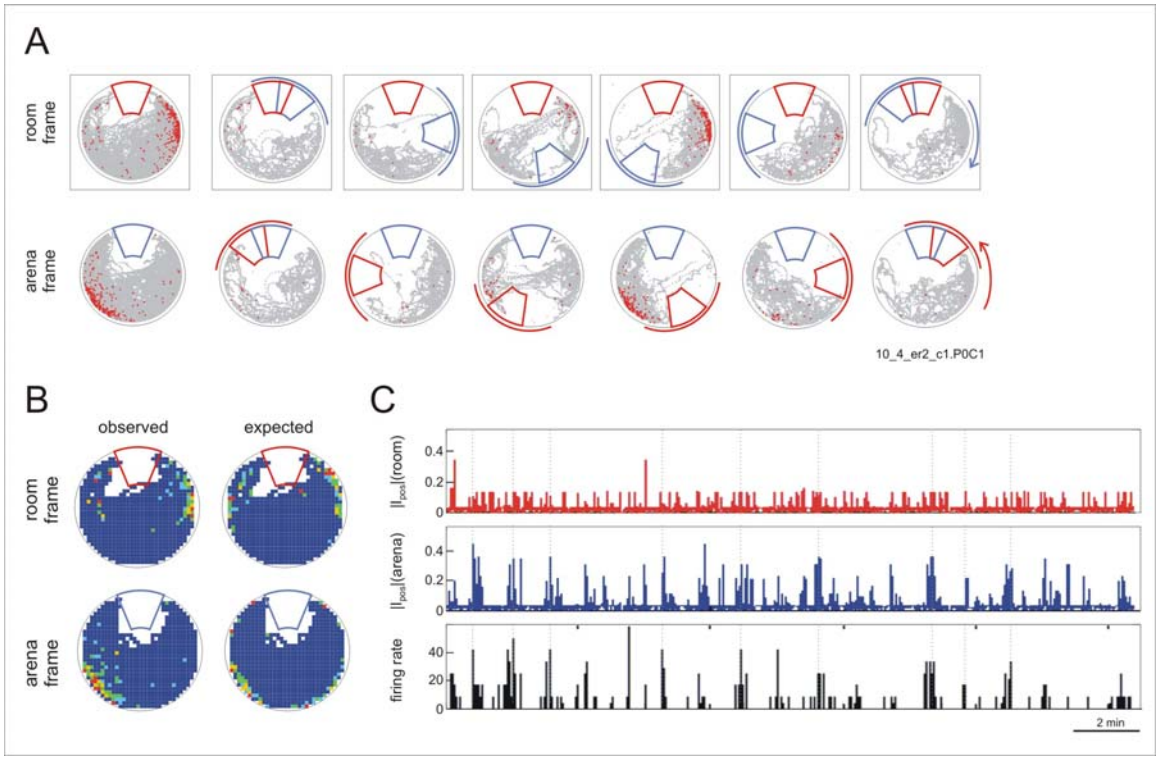

Figure 3. Characterization of spatial discharge of a cell that has firing restricted in both reference frames. A) Spike maps showing the distribution of action potentials in the two frames, and during different arena displacements in the room. B) Observed firing rate maps in the two spatial reference frames are shown to the left. Expected firing rate maps based on activity in the other frame are shown to the right. Figure suggests that arena frame firing largely predicts room frame activity (the correlation between the expected and observed spatial distribution of action potentials in the room frame was r=0.79). C) Absolute value of instantaneous positional information ($|I_{pos}|$) is shown for 117 ms intervals for both reference frames together with the firing rate during each interval. Firing of the cell coincides with increase in $|I_{pos}|$ in the arena frame, but no such tendency is observed in the room frame. This indicates that the cell's activity is related to arena-frame position, rather than room-frame position.

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3.1. Behavior in the room+arena+ place avoidance task

In this chapter we will study the rat's behavior in the room+arena+ place avoidance task, which requires the coordination of two simultaneously relevant but distinct spatial frames (the room frame and the arena frame). We will show that both continuously dissociated reference frames are used to organize the rat's behavior. We will demonstrate that the relative position of the two reference frames (the displacement of the arena in the room) as well as the direction of arena rotation affects the behavior. Finally, we show that coordinating the behavior according to the two reference frames requires intact hippocampus.

The rats avoided both shock zones.

We used four rats to study the learning of the room+arena+ place avoidance. After pre-training on a stable arena with a single shock zone, the rats were trained for the room+arena+ place avoidance on the rotating arena for eight days. During each of these eight days a single session of (room&arena)+ place avoidance on stable arena was followed by a session of room+arena+ place avoidance on the rotating arena. The rats learned to avoid both shock zones – the one defined in the reference frame of the room, as well as the one defined in the reference frame of the arena. The number of entrances to the shock zones during the room+arena+ place avoidance decreased with training (ANOVA with repeated measures, $F_{7,21}=7.82$, $p<0.001$, Fig. 2A). Newman-Keuls post-hoc comparison revealed that the number of entrances decreased significantly between the first and the second day (p's<0.001), and then did not change much (p's > 0.05). After eight days of training the rats entered the shock zones 5.75±2.25 times (mean±SEM) during a 16-minute session.

The between-trial memory in this task was assessed by measuring the time to the first entrance to the shock zones. The average time to the first entrance increased from 24.2±3.5 seconds during the first day, to almost six minutes (356.5±130.7 seconds) during the eighth training day. In six minutes the arena rotated six turns. The fact that the rats did not enter the shock zones for so long shows that they remembered shock zones from previous training trials.

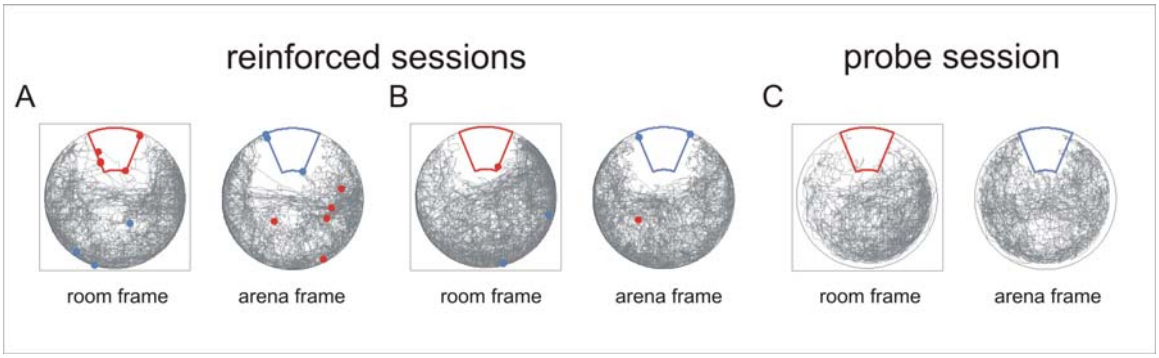

Figure 1. Behavior during three sessions of room+arena+ place avoidance task in well trained rats. The trajectory of the rat is shown by a gray line, position of the room shock zone is marked by a red line, and position of the arena shock zone is marked by a blue line. Red and blue dots indicate locations where the rat received foot-shocks after entering the room or arena shock zones. The diagram on the left shows the rat's trajectory in the coordinate system of the room, on the right the trajectory in the coordinate system of the arena is shown.
In sessions A and B the avoidance behavior was reinforced by a mild foot shock. Session C was a probe session without foot shock reinforcement, the avoidance behavior was still clearly present.

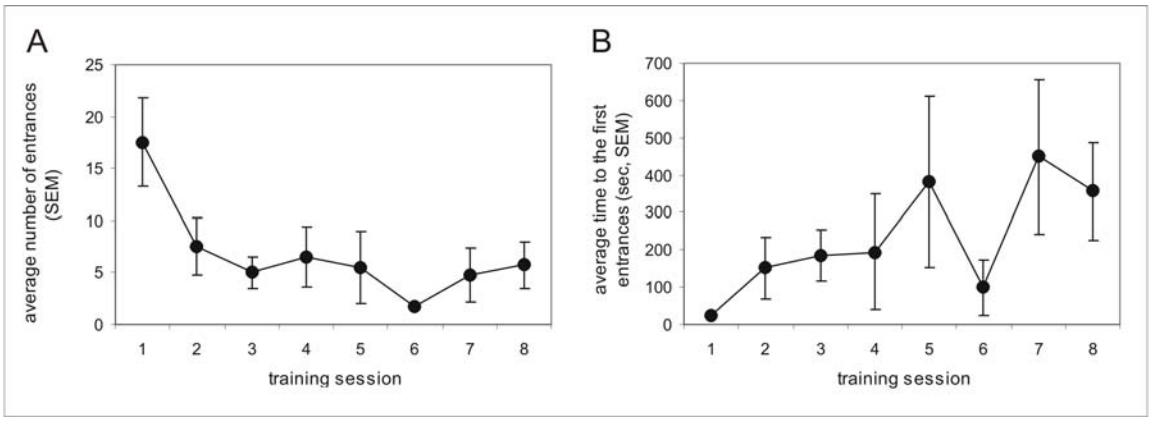

Figure 2. Learning the room+arena+ place avoidance task. Each session was performed on a separate day. A) Number of entrances to the shock zones decreased with learning (ANOVA with repeated measures, $F_{7,21}=7.82$, $p<0.001$). B) Time to the first entrance increased with learning showing the rats remembered the shock zones from previous learning trials (ANOVA with repeated measures, $F_{7,21}=1.29$, $p>0.05$)

Typical trajectories of well-trained rats during single sessions of room+arena+ place avoidance are shown in figure 1. The left plot shows trajectory of the rat in the reference frame of the room, the right plot shows the trajectory in the reference frame of the arena during the same recording session. The borders of the room and the arena shock zones are outlined by red and blue color respectively. Trajectory of the rat is shown in gray; places where foot-shocks were administered are marked by small dots. Red dots correspond to shocks delivered after the entrance to the room shock zone; therefore they appear on the borders of the room shock zone, but at random locations in the arena frame. Blue dots

correspond to shocks after entrances to the arena shock zone. During the session on figure 1A the rat entered the room shock zone five times, and the arena shock zone three times. During the session on figure 1B, the rat entered room shock zone once and arena shock zone twice.

The behavior in the room+arena+ place avoidance was also tested in three well-trained rats during probe trials with the shock turned off. During 20-minutes long probe trials the average number of entrances to the shock-zones was 6.7±4.1. The trajectory for one of the probe trials (Fig. 1C) shows clearly that even without the reinforcement in form of a shock, the rat respected the boundaries of both shock zones.

The rats responded to the displacement of the arena in the room.

A representation of the rat's position in the room frame, and a representation of the position in the arena frame could be stored and processed independently, or they could be somehow integrated. Integrated representation of the room and arena landmarks would be necessary to determine the relative displacement of the arena within the room. Therefore we asked whether the rat's behavior reflects the displacement of the arena in the room. The rats trained for the room+arena+ place avoidance task were tested during two test trials with different arena displacements. The arena was stable (did not rotate) during these test trials. During one test trial the arena shock zone overlapped with the room shock zone. During the second test trial the arena was rotated 180º, therefore the arena shock zone was opposite the room shock zone. The trajectory of six rats during these two tests is shown in figure 3A.

When the two shock zones were overlapping, the rats visited all the places of the arena except for the shock zones in the north. If the two shock zones were opposite to each other, the rats preferred to stay on the east side of the room, and never entered the west side (Fig. 3A). Whether the rats visited the west part of the room depended on the displacement of the arena in the room. This demonstrates that the relative position of the arena in the room affected the rat's behavior. Therefore we infer that not only information about both spatial reference frames is processed and represented in the rat's brain, but also the relative position of the two frames.

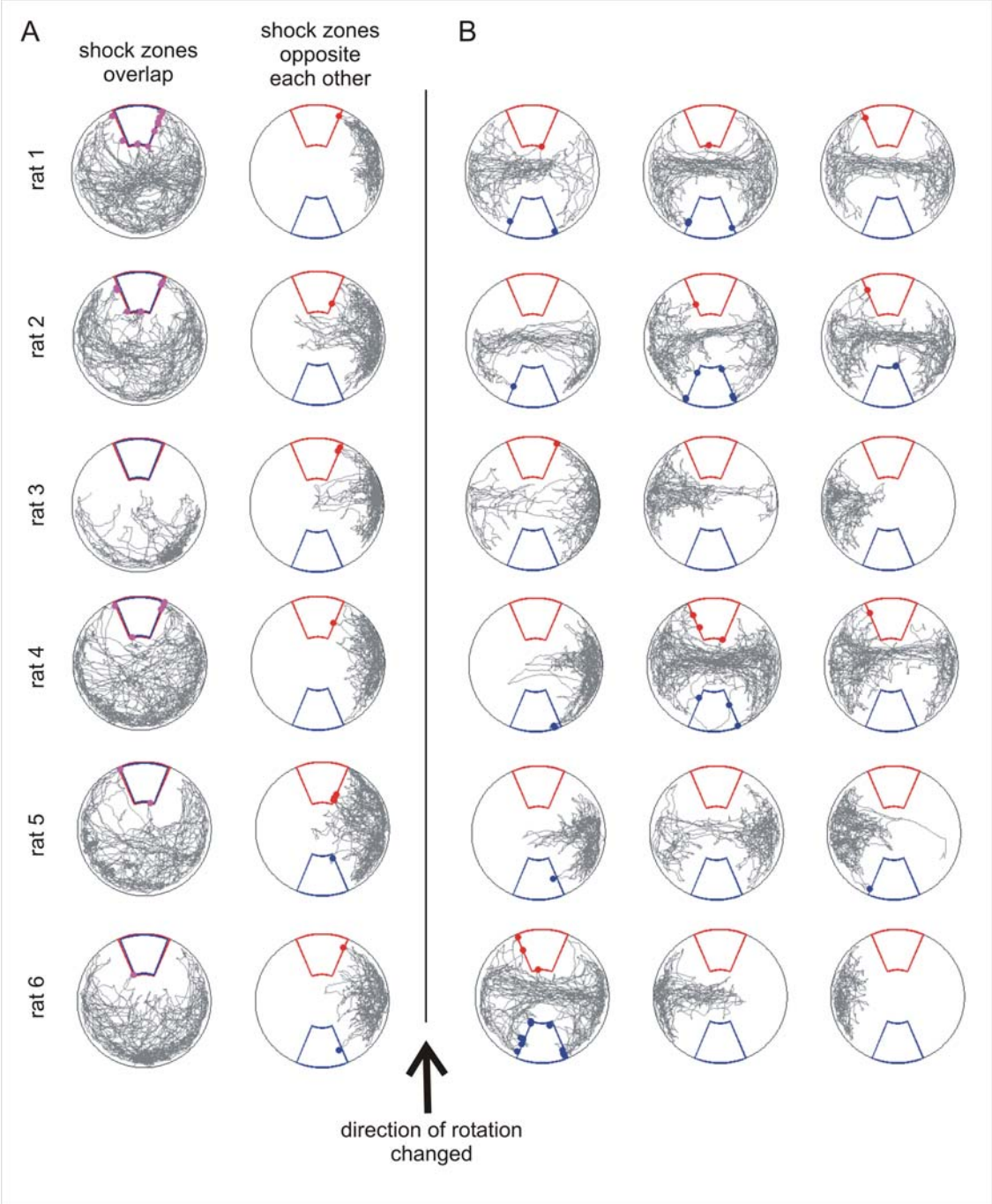

Figure 3. Six rats were trained to do room+arena+ place avoidance and then tested on the stable arena during two different displacements of the arena in the room. A) When the arena shock zone and the room shock zone overlapped, the rats walked all over the arena, except for the shock zone in the north. If the arena shock zone was placed opposite the room shock zone the rats only spent their time on the east side of the room. This shows that the relative displacement of the arena in the room influenced the rat's behavior. Therefore we infer that the representation of the room position and representation of the arena position are not completely independent, but are integrated.
B) When the rats were exposed to the arena rotating in the opposite (counter-clockwise) direction, and then tested on the stable arena with the two shock zones 180˚ apart, the preference for the east side of the room disappeared. This result shows that behavior was influenced by the direction of the arena rotation.

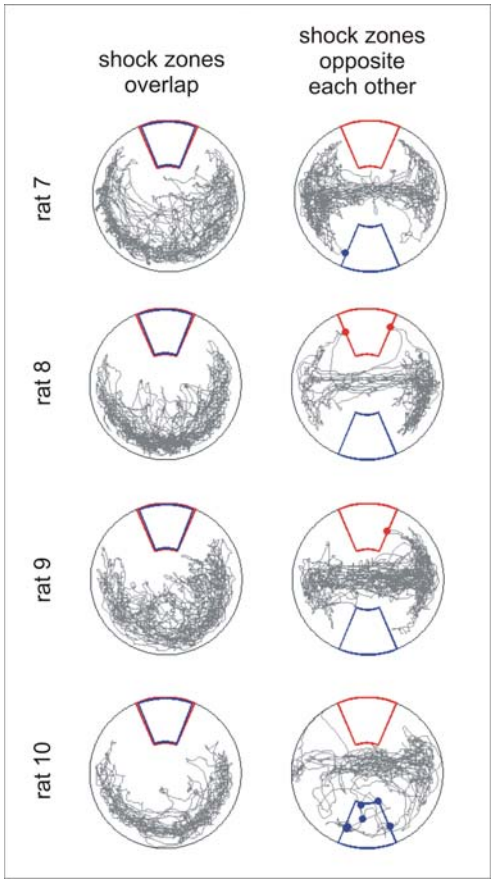

Figure 4. Four rats were trained in the room+arena+ place avoidance task until they reached asymptotic performance. These rats were exposed to both directions of rotation from the very beginning of training. When the arena rotation was stopped so that the room shock zone and arena-shock zone were opposite each other, the rats visited both the west and east part of the arena.

The direction of arena rotation affects the rat's behavior.

Why did all the rats prefer to stay on the same (east) side of the room when the two shock zones were opposite each other? We tested whether the direction of rotation during the training for the room+arena+ place avoidance task influenced the rats' behavior. During the training the arena was always rotated in the clockwise direction. If the two shock zones are opposite to each other and the arena is rotating clockwise, then the arena shock zone is moving toward the west part of the room. Therefore the east side of the room, the side preferred by the rats, is safer.

If this explanation is correct then changing the direction of the arena rotation should reverse the rats' preference from the east side to the west. We trained the same rats in the room+arena+ place avoidance task when the arena was rotating counter-clockwise. At the end of each training session the arena rotation was stopped, and the rat's behavior was

tested on stable arena with the two shock zones opposite each other. Within a few sessions all the rats walked to the west side of the arena and half of the rats stopped visiting the east side altogether (Fig. 3B). This confirms that the direction of arena rotation influences the rat's behavior. Finally, a separate group of four naïve rats was exposed to both directions of rotation from the very beginning of training. When these rats were tested with the two shock zones opposite to each other they visited both the west and east sides of the arena (Fig. 4).

Performance in the room+arena+ place avoidance task was impaired by unilateral hippocampal inactivation.

We also studied whether the room+arena+ place avoidance depends on intact hippocampal function (Fig. 5). Four rats were well trained for the room+arena+ place avoidance in a familiar environment. After training they were implanted with cannulea aimed at dorsal hippocampi (See chapter 2.1. for detailed methods). After recovery the rats were re-trained for the task. Their performance was tested one hour after injection of 1 µl volume of saline, or 5 ng of TTX in 1 µl saline into one hippocampus. Every rat received two injections of saline and two injections of TTX in random order. There was at least one-day interval between subsequent injections. The rats' performance was compared to sessions without injection.

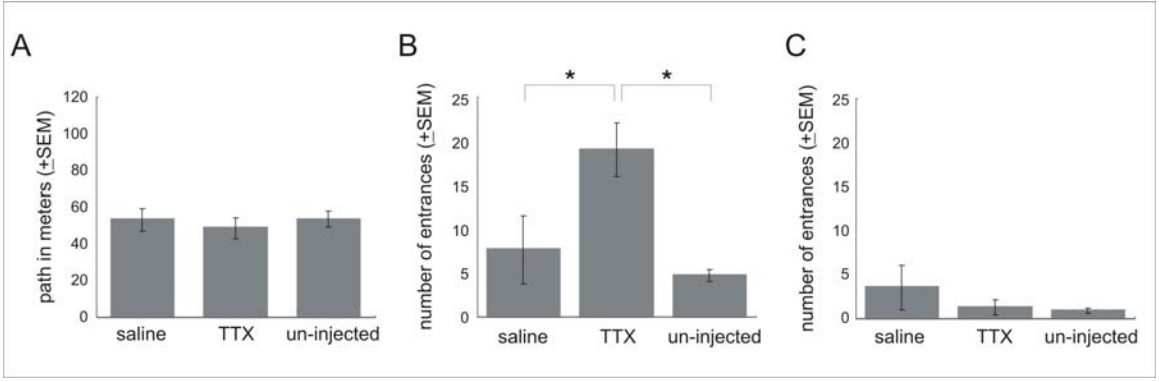

Figure 5. Four rats were well trained for the room+arena+ place avoidance task and then implanted with injection cannulea above hippocampi. Rats were tested an hour after unilateral hippocampal injection of TTX (5ng/1µl), or saline. Each rat was injected TTX or saline to left or right hippocampus in counterbalanced order during four different sessions. Each rat's performance was also tested without injection. A) TTX injection had no effect on overall path the rat walked during 15 minute session of room+arena+ place avoidance. B) TTX injection increased the number of shock-zone entrances during the room+arena+ place avoidance on rotating arena, but not during (room&arena)+ place avoidance on stable arena (C). (* p<0.05)

Unilateral TTX injection impaired performance in the room+arena+ place avoidance task on rotating arena (ANOVA with repeated measures $F_{2,6} = 9.12$, $p < 0.05$; Newman-Keuls post-hoc tests $p<0.05$, Fig. 5B). The unilateral hippocampal inactivation did not affect locomotion characterized by the distance walked ($F_{2,6} = 0.37$, $p = 0.71$, Fig. 5A), and it did not impair performance in (room&arena)+ place avoidance task on stable arena ($F_{2,6} = 1.16$, $p = 0.38$, Fig. 5C).

The room+ arena+ place avoidance on rotating arena is in many respects similar to the (room&arena)+ place avoidance on stable arena. The two tasks were performed in the same physical environment with identical landmarks, in both tasks the rats were reinforced by foot-shock of the same parameters to avoid shock zone(s) of the same size and shape. Yet, the former task was affected by unilateral hippocampal inactivation and the latter was not. What is the difference between the two tasks? On the rotating arena two distinct spatial frames are concurrently present and relevant, and are dissociated by the arena rotation. This is not the case for the task on stable arena. Behavioural impairment specifically in this task suggests that coordination of the two spatial frames may be affected by unilateral hippocampal inactivation. This is consistent with hypothesized role of hippocampus in cognitive coordination (Wesierska et al., 2005).

Analysis of behavior in the rats trained for the room+arena+ place avoidance task revealed that the rats processed their position relative to both spatial reference frames. Unilateral hippocampal inactivation experiment showed that the task requires intact hippocampal function. This makes the task a good paradigm to study the neuronal substrate underlying coordination of two distinct, simultaneously relevant representations.

3.2. Activity of hippocampal units during room+arena+ place avoidance

In this chapter we characterize spatial activity of single hippocampal CA1 complex-spike cells when the rats had to organize their behavior according to two continuously dissociated spatial reference frames. We will show that some cells responded according to the rat's position in the room, some responded according to the rat's position in the arena, and few cells responded in conjunction to both the arena and the room position. Then we will show that the quality of spatial organization of firing of the hippocampal CA1 complex-spike cells during the room+arena+ place avoidance is similar to that observed on a stationary arena, although a tendency for more compact firing fields can be seen under stationary conditions.

The previous chapter provided evidence that the position of the rat in the room frame, the position of the rat in the arena frame, as well as the displacement of the arena in the room are reflected in the rat's behavior during the room+arena+ place avoidance. Next, we investigated the role of the hippocampal neural network in processing of this information. The hippocampus was chosen for two main reasons: The role of hippocampal place cells in spatial information processing has been well characterized (Chapter 1.3). Moreover, previous work indicates that the hippocampus is directly involved in organizing spatial behavior with respect to two conflicting sets of cues on the rotating arena (chapter 3.1.; Kubik and Fenton, 2005; Wesierska et al., 2005).

Our first question was whether the discharge of hippocampal complex-spike cells was spatially organized in the two spatial reference frames during the room+arena+ place avoidance task. We recorded 224 cells from 11 rats. Ensembles of simultaneously recorded cells comprised up to 23 cells that were recorded from 5 different tetrodes at the same time. Of the recorded cells, 215 were classified as complex-spike cells (putative pyramidal cells), and 9 as theta cells (putative interneurons). Each ensemble of cells was recorded under two conditions – on the stable arena, and on the rotating arena. Of the 215 complex-spike cells 183 cells were active on the rotating arena (their firing rate was higher than 0.1 action potentials per second) and 157 cells were active on stable arena.

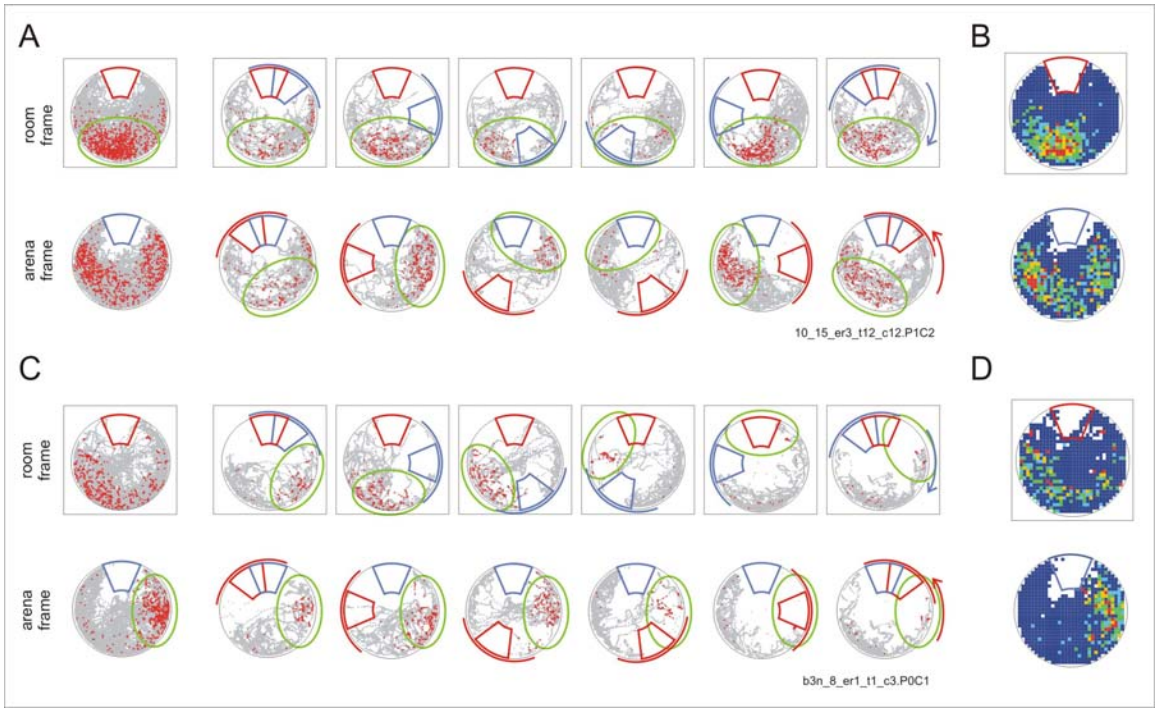

Figure 1. Frame-specific firing of individual place cells during room+arena+ place avoidance on the rotating arena. Locations visited by the rat (gray) and the locations where action potentials were emitted (red) are depicted in the room frame (upper row) and the arena frame (lower row). Frame-specific activity during the whole session is represented in the left-most column in A and C. The other six columns represent activity during different 60° ranges of the arena displacement within the room. The arc represents the span of the positions of the moving shock zone. A) This cell had a firing field opposite the room shock zone (red annulus sector) regardless of the arena's orientation in the room. Note that the action potentials were dispersed across the arena. B) Firing rate maps for the cell A in the room frame and arena frame.
C) This cell had a firing field close to the arena shock zone. If the rat visited this region, the cell fired, regardless of the arena's orientation in the room. Firing was dispersed in the room frame. D) Firing rate maps in the room frame and arena frame.

The two spatial reference frames are represented in the cells' discharge

Figure 1A shows the spatial distribution of action potentials of a complex-spike cell recorded during the room+arena+ place avoidance task. When shown in the reference frame of the room, the action potentials are clustered in the south part of the environment. In the reference frame of the arena the same action potentials are more scattered over the arena surface. The existence of the spatially restricted firing in the room frame and more random pattern in the arena frame indicates that the cell was responding according to the rat's position in the room. On different visits of the rat to the cell's firing field in the south part of the room, the arena was displaced differently and therefore the action potentials appear on scattered locations of the arena. This can be seen more clearly on

plots showing the location of spikes in the two reference frames for different angular displacements of the arena in the room (Fig. 1A, right). The cell fired in the south part of the room (marked by green ellipse in Fig. 1) regardless of the arena displacements. If the spikes are plotted in the arena frame they appear at different locations of the arena according to the arena displacement within the room.

Figure 1B shows an example of a different hippocampal complex-spike cell. The firing of this neuron was better organized in the reference frame of the arena than in the reference frame of the room, suggesting that the cell was responding according to the arena frame.

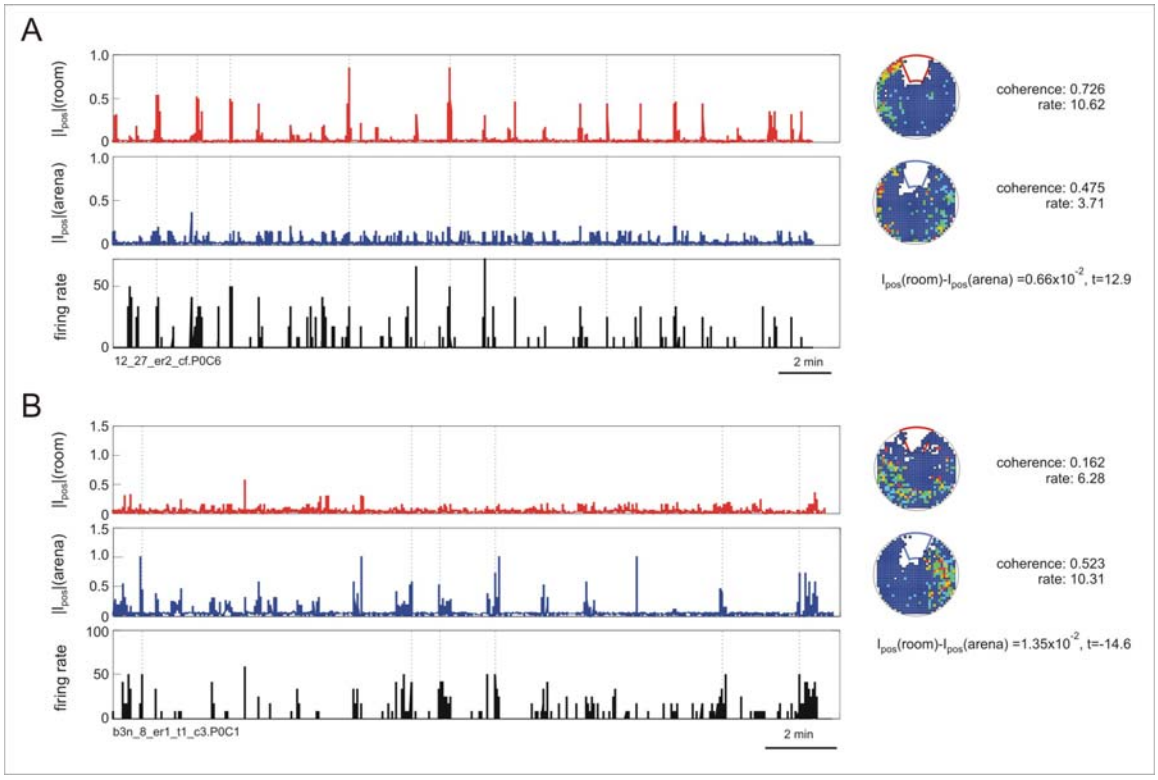

Figure 2. Quantifying frame preference using rate-map coherence and absolute value of instantaneous positional information ($|I_{pos}|$). $|I_{pos}|$ was computed during 117 ms time-intervals for both frames. Room-frame (red) and arena-frame (blue) $|I_{pos}|$ are shown together with the firing rate (black) of a cell. Instances of high firing rate of cell A were accompanied by increase in the room-frame $|I_{pos}|$, but not arena-frame $|I_{pos}|$. This indicates that the firing was characteristic for the position in the room, but not the arena. The room and arena rate maps are shown to the right. In the case of cell B, increase in the firing rate correlated with the increase in the arena-frame $|I_{pos}|$, but not room-frame $|I_{pos}|$. The firing of this cell was characteristic for the position on the arena, but not in the room.

We chose rate map coherence and absolute value of the instantaneous positional information ($|I_{pos}|$) to quantify positional firing of hippocampal cells (Chapter 2.6. reviews different methods to characterize spatial activity of place cells). While the coherence characterizes the spatial organization of firing averaged for the entire recording session, $|I_{pos}|$ can characterize firing during short time-intervals. Figures 2A and 2B show $|I_{pos}|$ as a function of time of a session for two hippocampal cells. The $|I_{pos}|$ in the room frame and the $|I_{pos}|$ in the arena frame were computed separately, and are shown along with the instantaneous firing rate of the cells. The room frame $|I_{pos}|$ of the cell in figure 2A increased at the moments when the cell's firing rate increased, however the $|I_{pos}|$ in the arena frame did not increase with increased discharge. Comparing $|I_{pos}|$ in the room frame and arena frame offers a way to statistically analyze frame preference of a cell. The difference between room frame $|I_{pos}|$ and arena frame $|I_{pos}|$ was computed and compared to zero by a t-test. Both room-preferring cells (96) and arena-preferring cells (87) were observed during rotation in similar numbers (test of proportions: $z = 0.67$, $p = 0.5$). In 64.5% of cells the t-test indicated statistically significant preference for one of the spatial frames.

Cells controlled by the conjunction of both spatial reference frames

In chapter 3.1. we have shown that the rat's behavior reflects the relative position of the arena in the room. Is the relative position of the arena in the room also reflected in the discharge of single hippocampal complex-spike cells?

As we discussed in chapter 2.6., the presence of firing fields in both reference frames is not sufficient to prove that both spatial frames modulate the activity of a particular cell. Because of unequal sampling of different locations during different arena displacements (see chapter 3.1.), "fake" firing fields may emerge in one of the reference frames. To determine the modulation of cell's activity by the two frames the firing rate maps must be supplemented by other methods of analysis. Each cell with firing fields in both reference frames was investigated using methods described in chapter 2.6. Briefly, for each spatial frame a firing rate map was created that would be predicted by firing in the other frame. These expected maps were compared with the observed maps. Additionally the $|I_{pos}|$ time series in both frames was compared. Based on this analysis we

identified nine cells with activity clearly modulated by both spatial frames. One example of such cell is shown in figure 3. Cells responding to both the room frame and arena frame may play a role in the coordination of the two spatial frames.

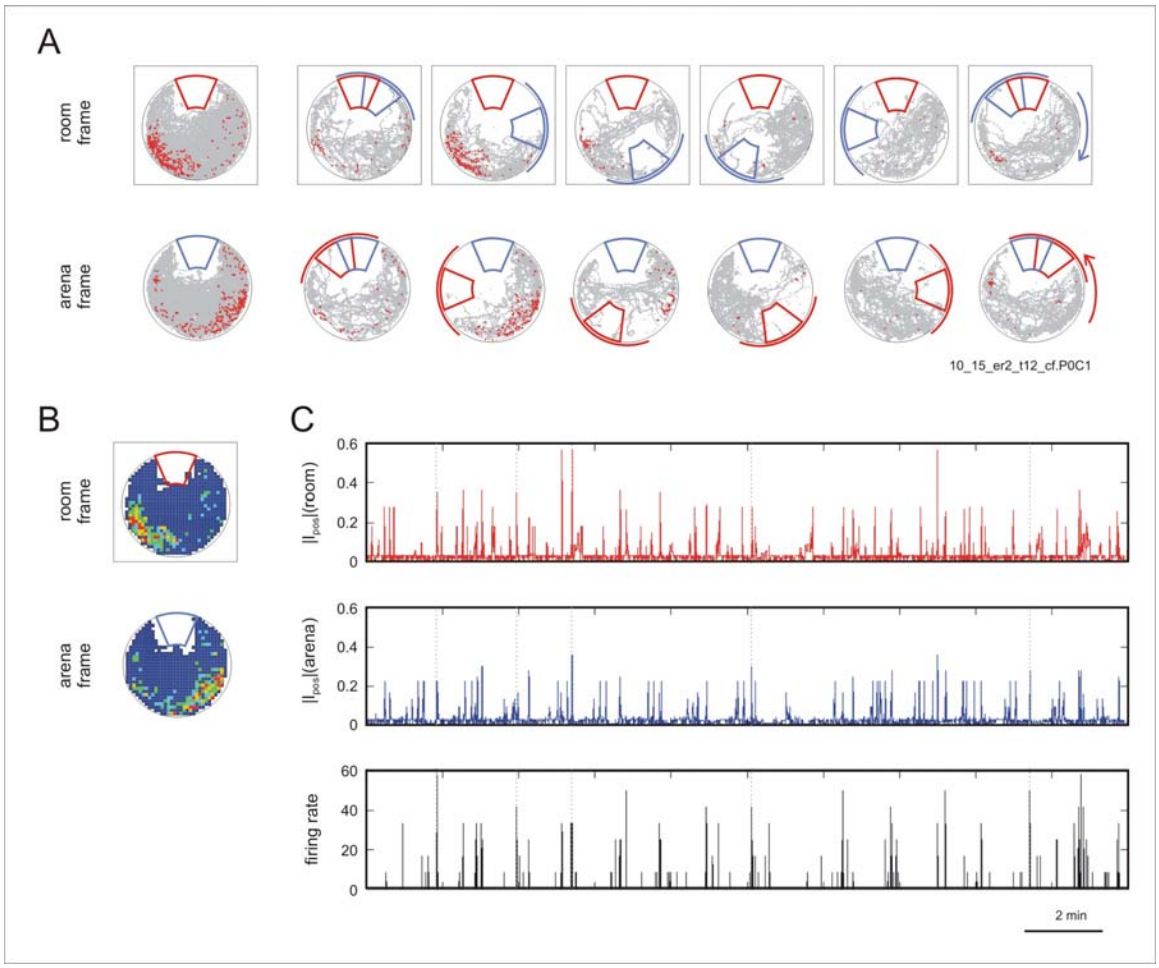

Figure 3. A cell with firing modulated by both spatial reference frames (one of the most convincing examples). This unit had a firing field in the southwest of the room and in southeast of the arena. The cell fired maximally when the arena shock zone was approximately 90º counter-clockwise from the room shock zone. A) Spike maps in the room frame – upper row, and the arena frame – lower row. Activity during different arena displacements in the room is shown. B) Firing rate maps of the same cell in the two frames. C) Time series of instantaneous positional information for the two spatial frames. Note that in both reference frames the value of $|I_{pos}|$ increased with increased firing rate.

In chapter 3.1. we reported that the rat's behavior was also influenced by the direction of arena rotation. To investigate the possible effect of direction of arena rotation on place cell activity 17 units were recorded from two rats during both clockwise and counter clockwise rotation of the arena. These preliminary data suggest that the place

cells were active in the same locations and had the same firing fields regardless of the direction of rotation (Fig. 4).

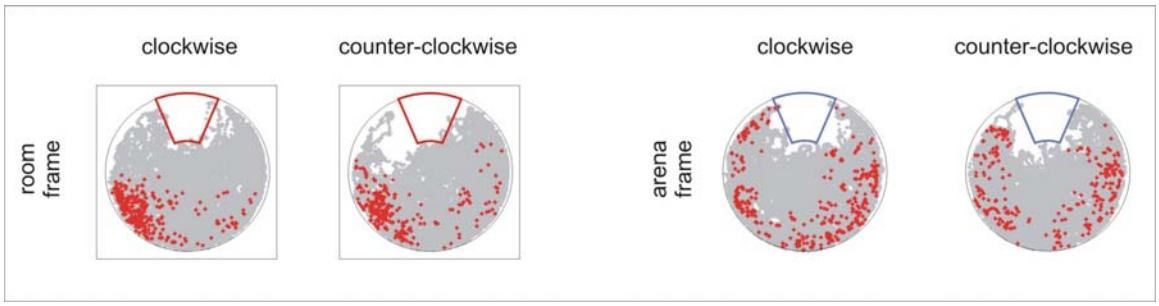

Figure 4. Similar spatial activity of a cell in room+arena+ place avoidance during clockwise and counter-clockwise rotation of the arena. The cell discharged in southwest of the room, its discharge appeared scattered on the arena surface, regardless of the direction of arena rotation.

Quality of spatial firing on rotating arena

Next, we quantitatively characterized the spatial organization of hippocampal pyramidal cell activity in room+arena+ place avoidance task on the rotating arena and compared it to activity on the stationary arena during (room&arena)+ place voidance. During rotation of the arena the spatial firing of a cell can be organized in the room frame or in the arena frame. A good organization of discharge in one frame of reference may lead to poor spatial discharge in the other frame of reference (see examples in fig. 1A and 1C). To avoid a bias toward low spatial organization, for each cell on the rotating arena the frame of reference with better-organized firing was analyzed separately from the other frame.

Average coherence observed during the stable session (0.48±0.02) was similar to the average coherence in the "preferred frame" that was observed during rotation (0.46±0.01) (t_{338}=0.90, p>0.05). The proportion of cells with well-organized firing fields was similar in the two conditions. The coherence ≥ 0.4 was used as an objective criterion for judging that a complex-spike cell had an excellent firing field, because this criterion matches the judgment of trained professionals. Of 157 complex-spike cells active during a stable session 103 cells (65.6%) had coherence higher than 0.4. Of 183 complex-spike cells active during rotation 121 cells (66.1%) had coherence higher than 0.4 in at least one spatial frame of reference. The properties of single cell firing under the stable and rotating conditions are compared in table 1. Spatial coherence, overall firing rate and

firing rate in the center of field were similar between stationary and rotating conditions. There was a tendency to lower information content, lower $|I_{pos}|$, and larger firing fields during rotating sessions compared to stationary sessions.

We also observed a tendency for cells in the preferred frame to have firing rate maps similar to the stationary conditions. However, the similarity of firing rate maps between stationary and rotating conditions was much lower than similarity between firing rate maps during two subsequent stationary sessions (Table 1). The similarity in ensemble activity between stationary and rotating conditions will be examined closer in chapter 3.5.

In summary, we showed that hippocampal pyramidal cells had spatially organized firing in the room+arena+ place avoidance task, and that both reference frames were represented in the place cell discharge, therefore in the next chapters we can ask, how the representations of the two spatial frames were coordinated.

Property	Stable	Rotating: preferred frame (comparison vs. stable)	Rotating: non-preferred frame (comparison vs. stable) (paired comparison vs. preferred frame)
Number of active pyramidal neurons recorded	157	183 χ^2=9.50, p<0.05	183 χ^2=9.50, p<0.05
Overall rate (AP/s)	0.86 $\pm$ 0.06	0.79 $\pm$ 0.06 t_{338}=0.78, p=0.44	0.79 $\pm$ 0.06 t_{338}=0.78, p=0.44
Spatial similarity to stable* (std units)	0.39 $\pm$ 0.026	0.16 $\pm$ 0.014 t_{213}=8.73, p<0.001	0.03 $\pm$ 0.010 t_{213}=15.43, p<0.001 t_{135}=11.42, p<0.001
average I_{pos} (bits)	0.022 $\pm$ 0.0015	0.013 $\pm$ 0.0009 t_{338}=5.42, p<0.0001	0.009 $\pm$ 0.0007 t_{338}=8.33, p<0.0001 t_{182}=10.19, p<0.0001
Coherence (std units)	0.48 $\pm$ 0.02	0.46 $\pm$ 0.01 t_{338}=0.90, p=0.37	0.32 $\pm$ 0.01 t_{338}=7.35, p<0.0001 t_{182}=15.74, p<0.01
Center rate** (AP/s)	9.68 $\pm$ 0.52	8.86 $\pm$ 0.61 t_{333}=1.00, p=0.32	5.67 $\pm$ 0.39 t_{331}=6.28, p<0.0001 t_{180}=9.62, p<0.0001
information (bits/AP)	2.13 $\pm$ 0.08	1.78 $\pm$ 0.06 t_{338}=3.57, p=0.0004	1.53 $\pm$ 0.05 t_{338}=6.60, p<0.0001 t_{182}=11.58, p<0.0001
Active proportion of arena	0.24 $\pm$ 0.01	0.31 $\pm$ 0.01 t_{338}=-4.71, p<0.0001	0.34 $\pm$ 0.01 t_{338}=-6.40, p<0.0001 t_{182}=14.11, p<0.01

Table 1. Discharge properties of recorded complex spike cells. Average and SEM values are given. *Spatial similarity was only analyzed in units that had spatially well-organized firing in at least one condition (coherence>0.4 was used as a criterion for well-organized firing.). **Only neurons with a firing field were analyzed.

(SpikeMap_ang.m; ChangeTS.m)

3.3. Cells active within a single session of room+arena+ place avoidance task preferentially represent the same spatial reference frame.

In this chapter we demonstrate that when a rat organized its behavior according to two simultaneously relevant but distinct spatial reference frames, there was a tendency among the cell recorded within the same session to preferentially represent the same spatial frame of reference. This suggests that hippocampal activity is organized into groups of coactive cells with shared function. We also present evidence that the preferred spatial frame was not hard-wired for a group of hippocampal cells, but the same ensemble of cells could encode different frames in different environments.

Simultaneously recorded cells tend to code for the same frame of reference

The central question of this work is how different representations are organized, how conflict between multiple concurrently relevant representations is avoided. One possible solution to this problem is to process only one representation within a neural network. In this chapter we study whether cells recorded during the same session of room+arena+ place avoidance shared preference for the same spatial reference frame.

Both room-preferring cells (96) and arena-preferring cells (87) were observed during rotation (test of proportions: $z=0.67$, $p>0.05$). Examples of recordings such as those in figure 1A and B suggest that the simultaneously recorded cells had a strong tendency to respond to the same reference frame. The spatial firing of the recording depicted in figure 1A was dominated by the arena spatial frame. Both I_{pos}, and rate map coherence showed a clear tendency of the ensemble to have firing organized in the arena frame. Figure 1B shows a room frame-preferring ensemble.

We analyzed the preferred frame of reference of pairs of cells that were recorded within the same sessions. We compared the number of cell pairs in which both cells coded for the same reference frame (concordant pairs) with the number of pairs in which the two cells coded for different reference frames (discordant pairs). Significantly more concordant pairs were observed (Fig. 1D, E, F). The significant tendency for concordant activity of simultaneously recorded cells was observed whether rate map coherence

($z=3.71$, $p<0.001$, Fig. 1E) or instantaneous positional information ($z=5.25$, $p<0.0001$, Fig. 1D,F) was used to assess the preferred frame of reference of the cells, and whether all active cells or active cells with statistically significant frame preference were used ($z=3.68$, $p<0.001$, Fig. 1F). Therefore we rejected the hypothesis that the simultaneously recorded cells are independently assigned to the two frames of reference.

In five rats we recorded at least one session of the room+arena+ place avoidance task with more than ten complex-spike cells active (firing rate>0.1 Hz) simultaneously. A preference for the two reference frames was studied in each of these five rats separately using the rate map coherence (Fig. 1C). For each session the reference frame preferred by the majority of cells was called the dominant frame. In three of the rats, the number of cells in the dominant frame was significantly higher than would be expected by chance.

Cells recorded from distant locations have concordant frame-specific responses

Next we asked whether cells across distant hippocampal locations are also likely to code for the same spatial frame of reference. We analyzed cell pairs recorded during the same sessions from different tetrodes implanted at least 400 μm apart. Whether the rate map coherence or instantaneous positional information was used to determine the frame preference, a significant tendency of the pairs of cells to code for the same frame of reference was observed (Fig. 1D, E, F). These analyses show that the tendency for concordant responses of simultaneously recorded cells was not limited to the cells recorded from the same tetrode. Therefore we can exclude potential imperfections in separating action potentials from different cells of the same tetrode as a source of the tendency for concordant responses. We can also conclude that the grouping of the cells with the same frame preference extends at least several hundred micrometers.

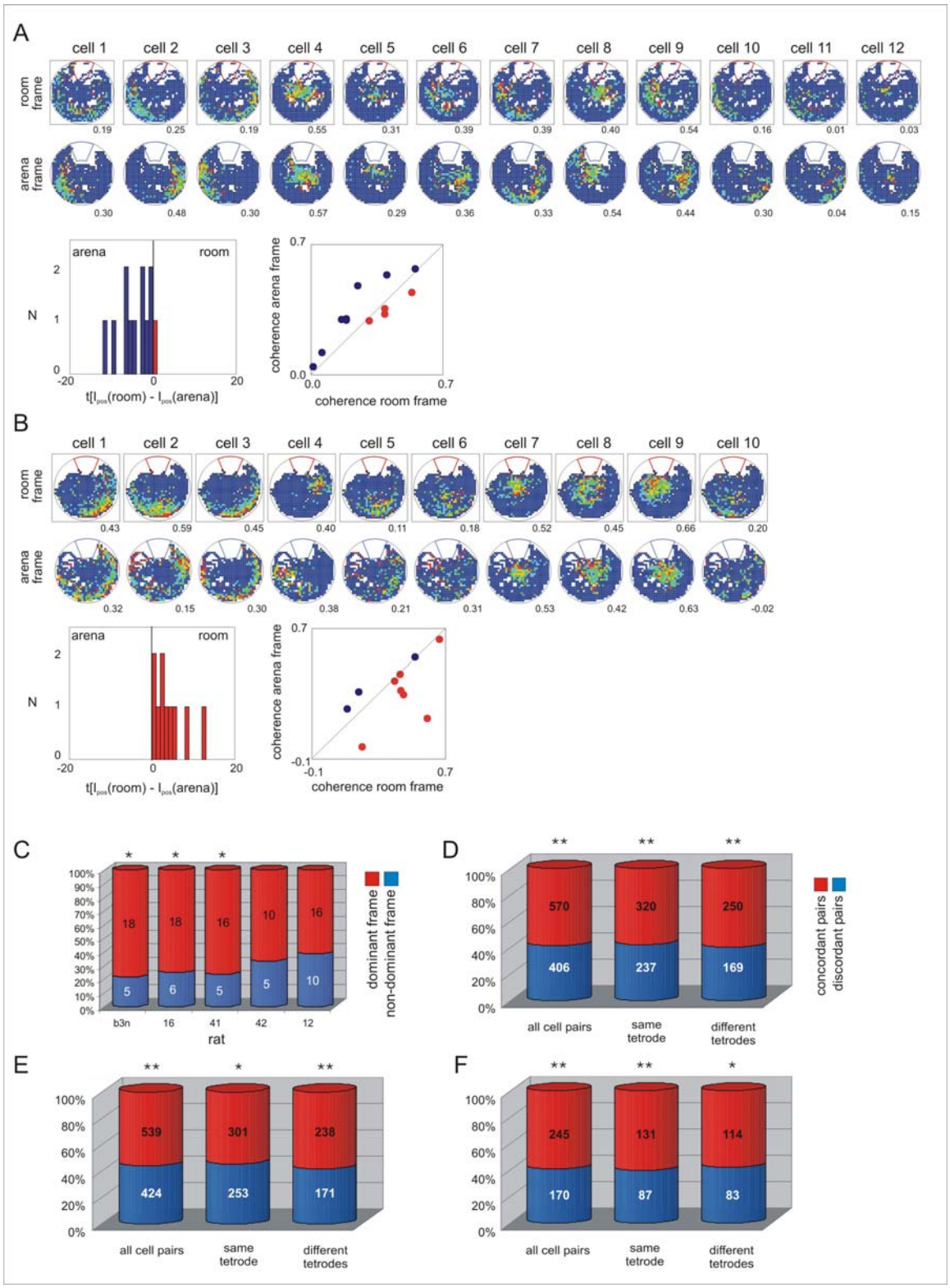

Figure 1. Most simultaneously-recorded cells tended to have the same frame preference. An example of an arena-preferring (A) and a room-preferring (B) ensembles from the same rat. The preference was quantified using absolute value of instantaneous positional information ($|I_{pos}|$) and rate map coherence. The difference between room frame and arena frame $|I_{pos}|$ for each cell indicates room preference in ensemble A and arena preference in ensemble B. Scatter plot of arena map coherence

and room map coherence also indicates the ensemble frame preference. The numbers bellow firing rate maps indicate rate map coherence.
C) Rats with more than 10 simultaneously recorded complex-spike cells active. For each session the reference frame preferred by the majority of cells was called the dominant frame, the reference frame preferred by fewer cells was called non-dominant frame. In three of the rats a significant tendency for the same frame preference within an ensemble of cells recorded within a single session could be detected (*p<0.05).
D, E, F) Pairs of simultaneously recorded cells tended to have concordant frame preference. For each cell pair we determined whether the preferred spatial frame was the same for the two cells (concordant pair) or if the two cells had different preferred frame (discordant pair). D) All cell pairs were analyzed using $|I_{pos}|$. The tendency for an excess of concordant cell pairs was observed when all cell pairs ($z = 5.25$; **$p<0.0001$), cell pairs from the same tetrode ($z = 3.52$; **$p < 0.0005$), or different tetrodes ($z = 3.96$; **$p < 0.0001$) were analyzed. The distributions of concordant and discordant cell pairs were similar whether the cells were drawn from the same or different tetrodes ($\chi^2 < 0.15$, $df = 1$, $p > 0.5$).
E) All cell pairs were analyzed using spatial coherence. The tendency for an excess of concordant cell pairs was observed when all cell pairs ($z = 3.71$, $p < 0.0005$), only cell pairs from the same tetrode ($z = 2.04$, $p < 0.05$) or only cell pairs from different tetrodes (rate map coherence: $z = 3.31$, $p < 0.005$) were analyzed.
F) $|I_{pos}|$ was used to assess the frame preference only in cells with a significant preference for one of the frames. The tendency for an excess of concordant cell pairs was observed when all cell pairs ($z = 3.68$, $p < 0.0005$), only cell pairs from the same tetrode ($z=2.98$, $p<0.005$) or only cell pairs from different tetrodes ($z = 2.21$, $p < 0.05$) were analyzed.

Ensemble preference is not hard-wired

Whether the preference for a particular reference frame is hard-wired was tested by recording the same ensembles during the room+arena+ place avoidance in two different environments. If the preference for a particular frame was hard-wired, then the frame preference of an ensemble should be the same in both environments. Three ensembles were recorded in two different rooms from one rat (Fig. 2A). Similar arenas were used in the two rooms. In one of the rooms the arena was surrounded by a black curtain, in the other room no curtain was used and the animal was exposed to the square ground plan of the room, and its light-blue walls. The time between recordings in two environments was between 1 and 6 hours. Within the same room, the cells of the same ensemble were likely to code for the same reference frame, confirming our previous observations (Fig. 2B). However, there was no tendency for the members of the same ensemble to have similar frame preference in two different environments (Fig. 2B). These results indicate that the frame preference was not preserved between the two environments; therefore we conclude that the preference of the ensembles is not hard-wired.

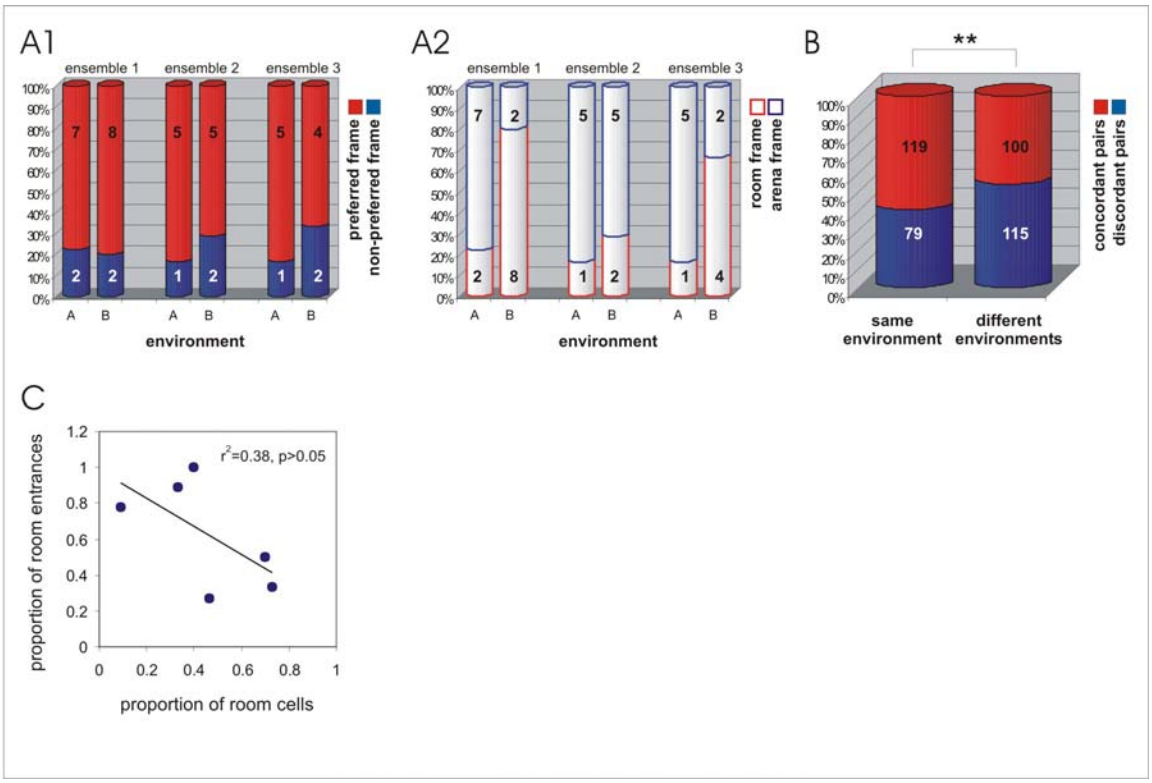

Figure 2. A change of frame preference in different environments. Three ensembles were recorded in two different environments (rooms). A1) Plotting the number of cells with a preference for the ensemble's preferred and non-preferred frame reveals that within each recording, the majority of cells preferentially responded to locations in the same reference frame. A2) Plotting the same data according to room- and arena-preference, reveals that the preferred ensemble frame in the two environments could be the same (ensemble 2) or different (ensembles 1 and 3). B) Ensemble cell pairs had an excess tendency to express concordant frame preferences within the same environment ($z = 2.84$; $p < 0.01$), but not between different environments ($z = 1.02$; $p = 0.31$; same versus different environment: $\chi 2 = 7.42$; $^{**}p < 0.01$).
C) Relationship between the hippocampal representation of the two reference frames and the rat's performance in the room+arena+ place avoidance task. Proportion of room cells in an ensemble seems to be negatively correlated with the proportion of entrances to the room shock zone, although the tendency was not significant. (Only recordings with more than one entrance were analyzed.)

Ensemble preference and rat's behavior

Is the tendency to respond to one of the reference frames reflected in the rat's behavior? Is the rat making more mistakes in the "underrepresented" frame of reference? We analyzed sessions of room+arena+ place avoidance in which at least 10 complex-spike cells were simultaneously recorded and the rat made at least two entrances to the shock zones. Because the rats were very well trained for the task, and rarely entered the shock zones, only six sessions met these criteria. In this small sample we observed statistically insignificant tendency for correlation between electro-physiologically

determined ensemble frame preference and behavior. Ensemble preference was measured as the proportion of room preferring cells in an ensemble. Room preferring cells were defined as cells that had higher room frame coherence than arena frame coherence. We observed a tendency for the proportion of room preferring cells to be negatively correlated with proportion of room shock-zone entrances (r^2=0.38, p>0.05, Fig. 2C). The better the room frame was encoded, the less "mistakes" the rat made in the room frame.

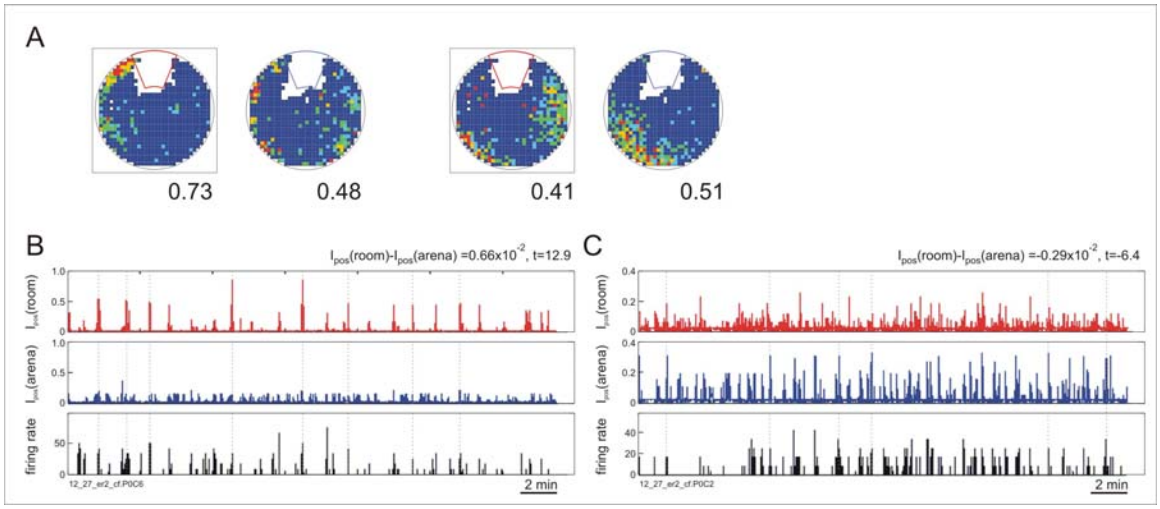

Figure 3. An example of a pair of cells discordant frame preferences recorded in one session. A) Firing rate maps of cells in room+arena+ place avoidance task. Numbers show spatial coherence for each firing rate map. B) and C) Time series of $|I_{pos}|$ of two of the cells indicate opposite frame preference.

Cells with discordant frame preference

Throughout this chapter we studied and emphasized the tendency of simultaneously recorded cells to have firing organized in the same reference frame. Now we will turn our attention to the fact that some of cell pairs had discordant responses (Fig. 3). We compared the firing rate, spatial coherence and other properties of the cells in the dominant and non-dominant spatial frames and we did not detect significant difference in any of these properties (Table 1). It may appear, as if the existence of the discordant cell pairs contradicted the main finding of coherent responses of hippocampal ensembles. When cells with both dominant and non-dominant responses were present in an ensemble, where they not interfering with each other's function? One possibility to prevent interference is to separate the potentially conflicting cell activity in time. The

hypothesis that timing of cellular activity may play a role in organizing responses of cells in the two spatial frames was analyzed and supported. This is the main topic of the following chapter. It shows that timing of activity contributes to organizing discharge of hippocampal cells into functionally related groups.

In summary, we showed that cells recorded during the same session of the room+arena+ place avoidance are likely to have firing organized in the same reference frame. This suggests that groups of cells with common function are preferentially active together. Organizing cells into distinct functionally-defined groups may provide a way of avoiding interference between competing representations. We observed that the frame preference of cells was not hard-wired – the hippocampal ensembles had flexibility to code for different reference frames in different environments.

Property	Dominant frame cells	Non-dominant frame cells (comparison)
Overall rate (AP/s)	0.80 $\pm$ 0.06	0.87 $\pm$ 0.16 (t_{164}=-0.46; p>0.05)
Coherence (std units)	0.46 $\pm$ 0.02	0.45 $\pm$ 0.02 (t_{164}=0.11; p>0.05)
Δ coherence (preferred – non-preferred)	0.13 $\pm$ 0.01	0.14 $\pm$ 0.02 (t_{164}=-0.31; p>0.05)
Average I_{pos} (bits)	0.012 $\pm$ 0.001	0.014 $\pm$ 0.002 (t_{169}=-0.83; p>0.05)
$t\left(I_{pos}preferred - I_{pos}nonpreferred\right)$	4.49 $\pm$ 0.38	5.19 $\pm$ 0.78 (t_{169}=-0.89; p>0.05)
Center rate* (AP/s)	7.67 $\pm$ 0.67	9.74 $\pm$ 1.48 (t_{162}=-1.47; p>0.05)
Information content (bits/AP)	1.66 $\pm$ 0.07	1.84 $\pm$ 0.14 (t_{164}=-1.32; p>0.05)
Active proportion of arena	0.33 $\pm$ 0.01	0.32 $\pm$ 0.03 (t_{164}=0.35; p>0.05)

Table 1. Comparison of properties of cells in dominant and non-dominant frames. *Only cells with firing fields were analyzed.

3.4. Organization of neuronal discharge on timescales of milliseconds and seconds is related to spatial response properties of hippocampal neurons.

In this chapter we analyze the temporal organization of hippocampal discharge and its potential role in coordinating different representations. First, we studied discharge on the timescale of the hippocampal theta rhythm (6-12 Hz). On the stable arena we observed that the cells firing within a few milliseconds had more similar firing fields than cells firing tens of milliseconds apart. During the room+arena+ place avoidance on the rotating arena, the cells coding for the same spatial frame of reference were more likely to fire together within a few milliseconds. We also studied activity of neurons on timescale of seconds. Again, we observed that the place cells with positively correlated activity had more similar firing fields. Observed temporal organization of hippocampal discharge may provide mechanisms to prevent temporal overlap and interference between functionally related groups of neurons.

Theories about information processing in the nervous system suggest that action potentials emitted by neurons participating in the same representation should be coordinated in time (Abeles, 1991; von der Marlsburg, 1981). In the hippocampus, the field potential oscillations with frequency of 6-12 Hz (theta rhythm) are implicated in organizing neuronal discharge (Buzsáki and Eidelberg, 1983; Fox et al., 1986; O'Keefe and Recce, 1993; Klausberger et al., 2003), and activity-dependent synaptic plasticity (Pavlides et al., 1988; Otto et al., 1991; Hölscher et al., 1997; Hyman et al., 2003). We studied whether different spatial representations are organized on the physiologically relevant timescale of the hippocampal theta rhythm.

Temporal organization of activity on timescale of theta rhythm

First, we characterized the temporal organization of hippocampal firing on the stable arena during (room&arena)+ place avoidance. Cross-correlations of action potentials from pairs of hippocampal pyramidal cells were computed. Pairs of cells that were coactive and provided at least 100 counts within 128 ms on a cross-correlation plot

were further analyzed. Cross-correlations often showed a tendency of one cell to discharge with a characteristic time delay after another cell (Fig. 1A).

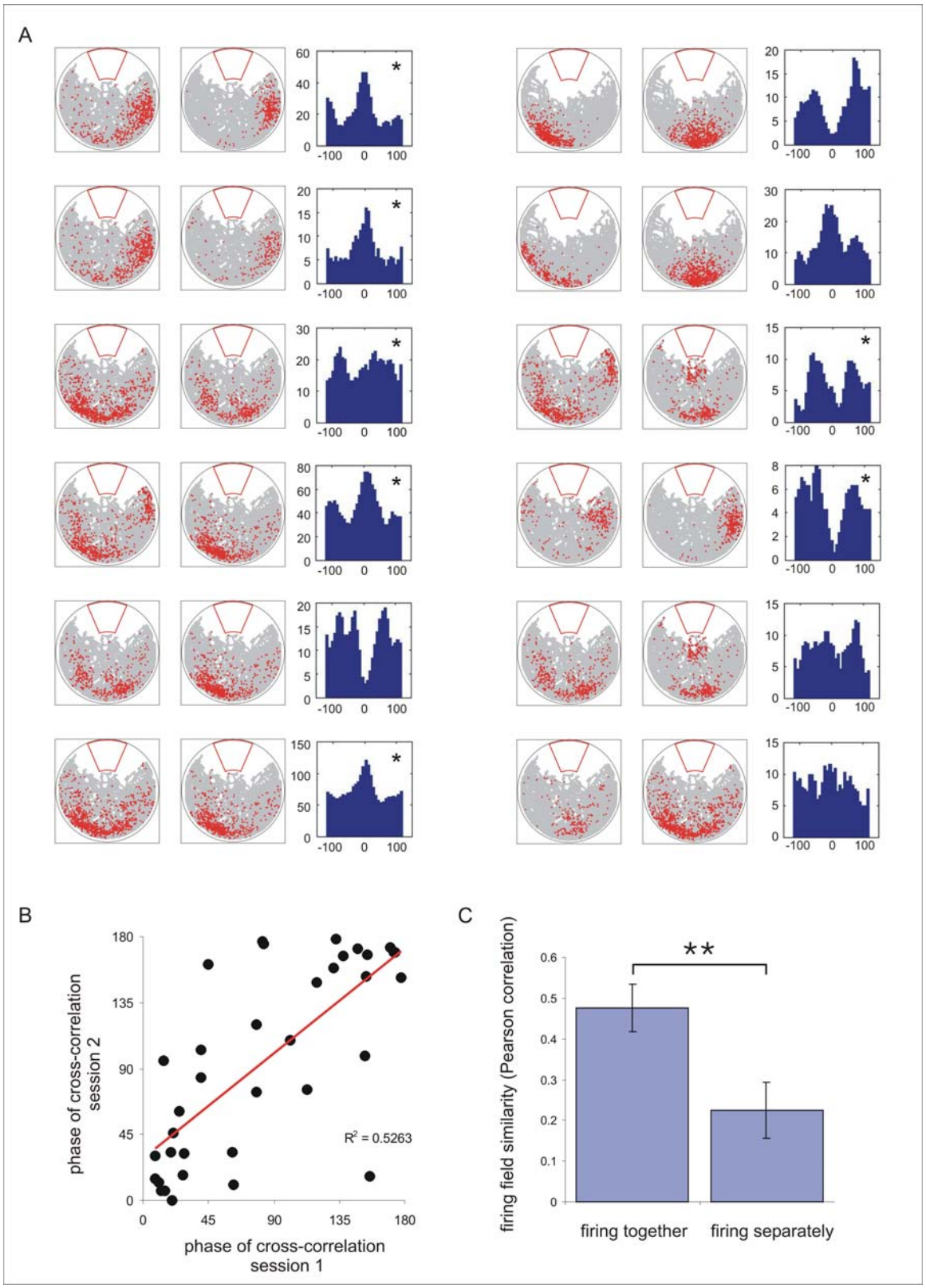

Figure 1. Cross-correlation of place cell firing on the stable arena during the (room&arena)+ place avoidance task. A) Examples of cross-correlations for pairs of cells are shown together with spike maps of the same cells. Strong modulation by theta rhythm is seen in most, but not all of the cross-correlation plots. Some cells discharge close in time, as is indicated by cross-correlation peak close to zero, other cells discharge with characteristic time delay. Cell pairs marked by an asterisk were recorded from different tetrodes. B) The cross-correlation was typical for a cell pair and was preserved between experimental sessions (r^2=0.53, p<0.001). C) Cells firing closer together in time have more similar firing fields. If the cross-correlation had strong theta modulation and the phase shift was between 0 and 45 degrees the cells were classified as firing together. If the phase of modulation was between 135 and 180 degrees the cells were classified as firing separately. The similarity of firing fields was assessed by correlation of firing rate maps. The tendency of cells firing together to have more similar firing fields was significant (t_{50}=2.78, p<0.01).

Another notable feature of most cross-correlation plots was a strong modulation at the timescale of theta rhythm. If there were multiple peaks on cross-correlograms, the distance between them often corresponded to period of theta oscillations (100-120 ms). Activity of pairs of cells relative to the ongoing EEG theta is illustrated in figure 2. The plots 2B and 2D illustrate that activity of CA1 pyramidal cells is modulated by theta rhythm. The cells' firing appears preferentially on one phase of theta. The spikes of two different cells may appear on the same theta cycle, leading to a cross-correlation peak close to 0 ms (Fig. 2C and 2D). Alternatively, the spikes of two cells may appear preferentially on different theta cycles, leading to cross-correlation peaks at around ± 100 ms, and small counts at 0 ms (Fig. 2A and B).

To characterize the timing of action potentials emitted by two cells, the cross-correlation was smoothed by averaging across three neighboring 8 ms time-bins. The time of the cross-correlation peak is one of the parameters used to characterize temporal relationship in cells' discharge. However, use of this parameter is limited because multiple peaks of similar amplitude were often observed in cross-correlation plots (Fig. 1, Fig. 2). Presence of multiple peaks could lead to measuring very different peak times in similarly looking cross-correlations. Therefore the phase of the theta modulation was used as an alternative measure, less sensitive to relative differences in amplitudes of multiple peaks. The Fourier transformation of the cross-correlations was performed, and phase of theta modulation was determined from the Fourier coefficients. The cross-correlation for the same cell pairs was stable across two subsequent recording sessions of the (room&arena)+ place avoidance (Fig. 1B).

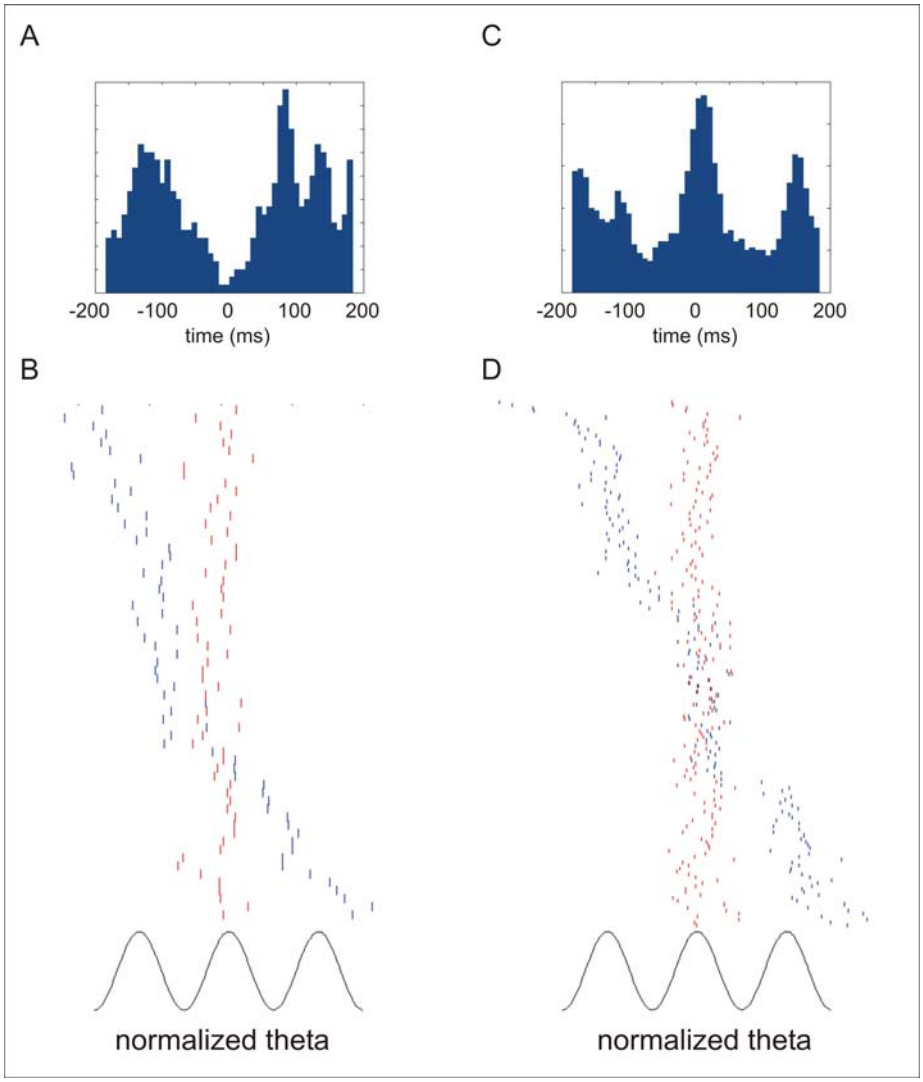

Figure 2. Modulation of activity of cell pairs by theta rhythm. A) Cross-correlation of two cells that rarely discharged within few milliseconds, but preferred to discharge 80-120 ms apart. B) Action potentials of the same two cells as in A) drawn relative to the phase of the EEG theta rhythm. Each row depicts a pair of action potentials that occurred within 200 ms. Action potentials of the two cells are shown in red and blue respectively. Data are organized according to the time between two action potentials. Both cells discharged predominantly on the positive phase of the theta rhythm. The blue cell rarely discharged on the same theta cycle as the red cell, but often discharged during the preceding or the following cycle. C) Cross-correlation of two cells that discharge preferentially few milliseconds from each other. D) Action potentials of the same two cells as in C) drawn relative to the phase of the theta rhythm. Both cells fired preferentially on the positive phase of theta. They often fired within the same theta cycle.

To study theta modulation of neuronal discharge, hippocampal EEG during the interval ±200 ms before and after each action potential was analyzed. Fourier transformation of the EEG signal was performed, and theta score was computed by dividing the power in the theta range (6-10 Hz) by the total power (1-1000 Hz). Time intervals with theta score bigger than 0.4, which indicated strong theta modulation, were further analyzed. The EEG signal was filtered between 5-12 Hz using Chebyshev type 2 filter, and phase of the theta at the moment of each action potential was determined using Hilbert transform.

Cells firing together on the theta timescale have similar response properties

We studied whether the characteristic timing of cell pair discharge was related to the signal that the cells presumably represent during (room&arena)+ place avoidance. The cell pairs were divided into two groups based on phase of modulation of cross-correlogram. One group included those cells that discharged together (phase of modulation $< 1/2\pi$) and the other group included cell pairs firing separately (phase of modulation $> 3/2\pi$). The similarity between the firing fields of the two cells was assessed by the correlation of the firing rate maps. We observed, that the cells that fired close together in time had more similar firing fields ($t_{50}=2.78$, $p<0.01$; Fig. 1C). This observation is consistent with the notion that cells participating in the same representation have stronger tendency to fire together and cells coding for different mental objects should have firing separated in time. It is also consistent with previous reports of Dragoi and Buzsáki (2006), and Itskov et al. (2008). Notice, that in spite of this overall tendency of cells with more overlapping fields to fire together in theta timescale, cell pairs with similar spatial overlap could have quite different cross-correlation plots (see top two cell pairs in the right column of figure 1A). Examples like this strongly suggest that similarity between firing fields is not the only determinant of temporal coordination of cell activity.

Next, we studied whether the representations of the two reference frames during the room+arena+ place avoidance task on the rotating arena are organized on the timescale of the theta rhythm. The cross-correlations for the cell pairs with firing better organized in the same frame or different frames of reference (concordant and discordant cell pairs respectively) were analyzed. Only cell pairs with theta modulated cross-correlation were studied. The cells with concordant responses were likely to fire close together in time ($\chi^2=32.4$, df=6, $p<0.001$; Fig. 3A). This tendency was preserved when cell pairs from the same tetrode or different tetrodes were analyzed separately. Such tendency was not observed in cells with discordant responses ($\chi^2=2.6$, df=6, $p>0.05$; Fig. 3B). These results indicate that groups of cells with the same frame preference have discharge coordinated in time on the theta timescale.

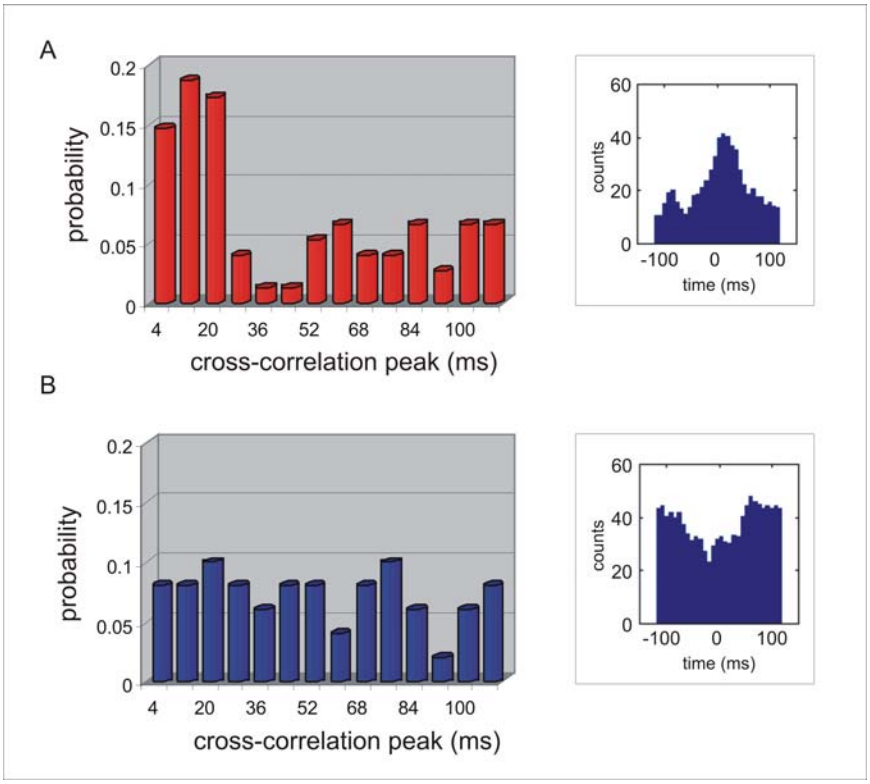

Figure 3. Cross-correlation of place cell firing on a rotating arena during the room+arena+ place avoidance task. A) Cell pairs with concordant responses (both cells had firing better organized in the same reference frame). Cells were more likely to discharge within 24 milliseconds (χ^2=32.4, df=6, p<0.001). To the right is an example of cross-correlation of one of the concordant cell pairs. B) Cell pairs with discordant responses (the two cells had firing better organized in different reference frames). No significant tendency to discharge within few milliseconds was observed (χ^2=2.6, df=6, p>0.05). To the right is an example of cross-correlation of a discordant cell pair. Cross-correlograms with more than 100 counts in the ±128 ms time window were used.

Coordination of discharge at the timescale of seconds

After we characterized firing at the timescale of the theta rhythm, we performed a similar analysis at the timescale of seconds. This is the timescale for mental phenomena as variable as selective attention, motor planning, or multistable perception of ambiguous images (Struber et al., 2000; Nakatani & van Leeuwen, 2005). Modulation of hippocampal activity at this timescale has been suggested by Olypher et al. (2002).

During (room&arena)+ place avoidance on a stationary arena we analyzed cell pairs with overlapping firing fields. Five-second intervals of data were analyzed, which corresponds approximately to time spent by a rat in a firing field (Fenton and Muller, 1998). Only those intervals were used when both cells were expected to fire with the rate of at least one spike per second, that is above average firing rate of hippocampal complex spike cells.

First step of our analysis revealed that the correlation between the firing rates of cells was positively related to the similarity between their firing rate maps ($r=0.56$, $p<0.001$, Fig 4A1). This is not a surprising result; it shows that cells with highly overlapping firing fields will fire together more often than cells that have smaller overlap between their firing fields. The same (even slightly stronger) tendency was observed when the same analysis was performed with simulated data ($r=0.76$, $p<0.001$). In the simulation the firing of a cell during each time interval was determined solely by the average firing derived from the firing rate map. In the simulated data fine temporal organization of discharge was disrupted without disrupting the firing rate maps, which remained identical to the recorded data (Fig 4A2).

Spatial-temporal covariance in place cell firing

Fenton and Muller (1998) demonstrated an excess variance in cell discharge during different visits of the rat to a cell's firing field. During one visit to the firing field a cell can discharge many action potentials; while during a different visit with a very similar trajectory the cell may not fire at all (Figure 3 in chapter 2.4. provides an example of this phenomenon). This overdispersion in place cell firing was theoretically studied by Olypher et al. (2002). We asked whether the excess variance in place cell firing between different visits to a firing field is related for pairs of cells, whether the activity of cells co-varies.

We standardized the firing rates at each time interval by the average firing rate characteristic for the rat's location during that time interval (see chapter 2.4. for detailed methods). This standardized firing signifies how much more or less a cell fired during each time interval compared to its expected firing based on the average firing rate at a given location. We asked whether the deviation of firing from the expectation correlates for a pair of cells. If one cell fired more than expected, did the other cell also fire more than expected; and vice versa? Figure 4B shows examples of three pairs of cells with overlapping firing fields. The scatter plots of standardized firing for pairs of cells are shown on the bottom. Each dot on these scatter plots represents a single five-second interval when both cells were expected to discharge at a rate higher than 1 spike/sec. The

correlation coefficient that characterizes the tendency of a cell pair to co-vary their firing is called spatial-temporal covariance.

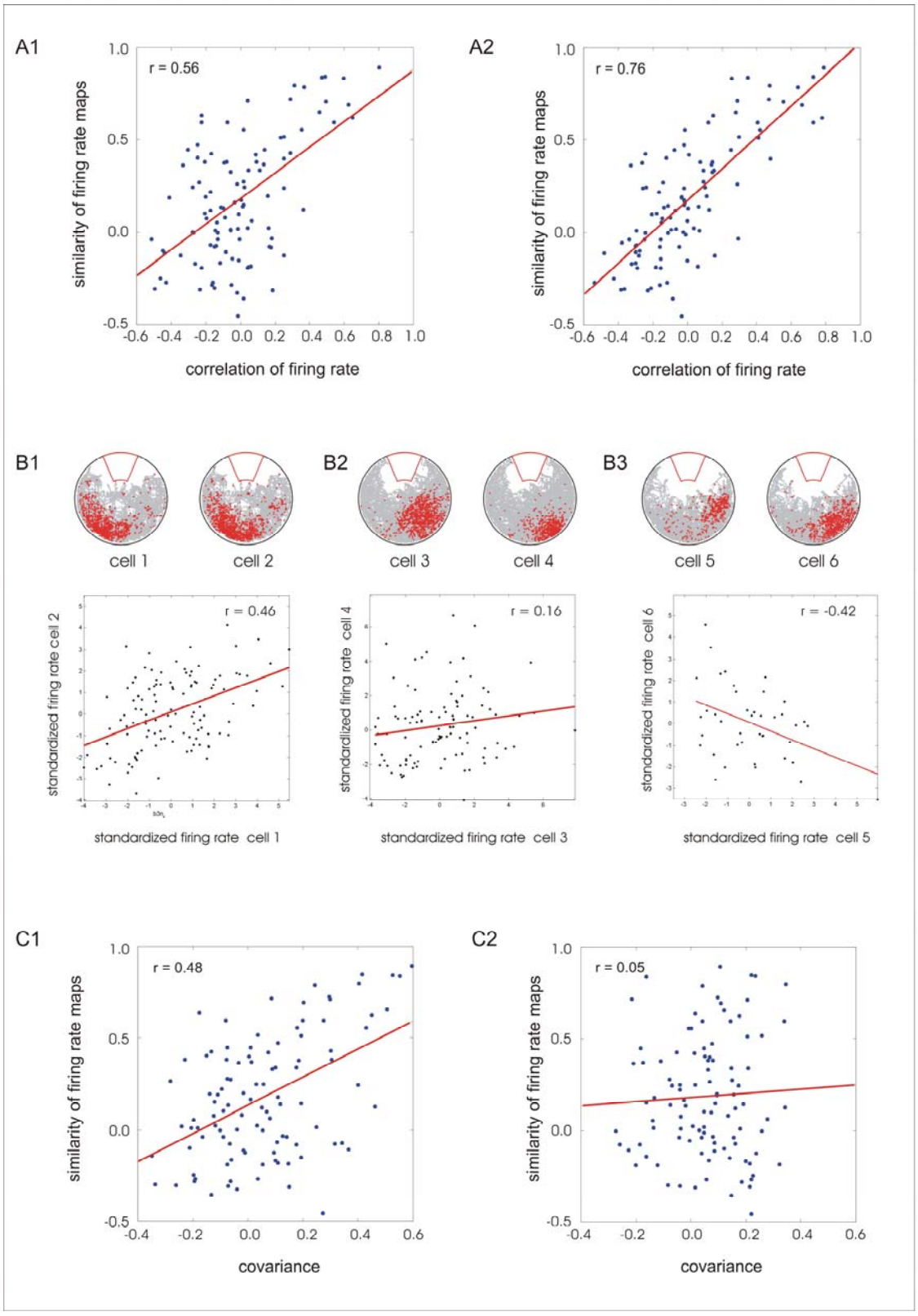

Figure 4. Characterizing temporal coordination of cell firing on timescale of seconds.
A) Relationship between firing rate of cells during five-second intervals and the firing field similarity. A1) Analysis of observed data: The more similar firing fields are, the stronger is the firing of the two cells correlated. A2) The same analysis as in A1 was performed using simulated data, which preserved the spatial characteristics as the recorded data (the same firing rate maps), but did not preserve temporal organization of firing. The tendency of the cells with better overlapping firing fields to discharge closer together in time appears also in the simulated data.
B) The spatial responses of three pairs of cells are show on the top. In the bottom row the covariance between the cells of each pair is shown. To compute the co-variance, the five-second intervals when the rat was in firing fields of both cells were analyzed. For each of these intervals the observed number of spikes was standardized by the number of spikes expected based on the trajectory of the rat. This standardized firing rate provides a measure (in units of standard deviation) how much more or less the cell fired compared to the expected average firing. Each dot characterizes one five second interval. The correlation of the standardized firing rates is called spatial-temporal covariance. Cells 1 and 2 varied their activity together, when one cell fired more than expected the other cell also had a tendency to fire more than expected, their covariance was 0.46. Cells 3 and 4 had lower covariance (0.16) and the covariance of firing of cells 5 and 6 was negative (-0.42), suggesting that when cell 5 fired more than expected, cell 6 fired less than expected.
C) Relationship between spatial-temporal covariance and the firing field similarity. C1) Analysis of observed data: The more similar firing fields were, the higher spatial-temporal covariance in cells' activity was observed. C2) The same analysis as in C1) was performed using simulated data in which the average firing rates and firing field locations of the cells were preserved. The tendency of the cells with overlapping fields to co-vary in their firing was no longer present.

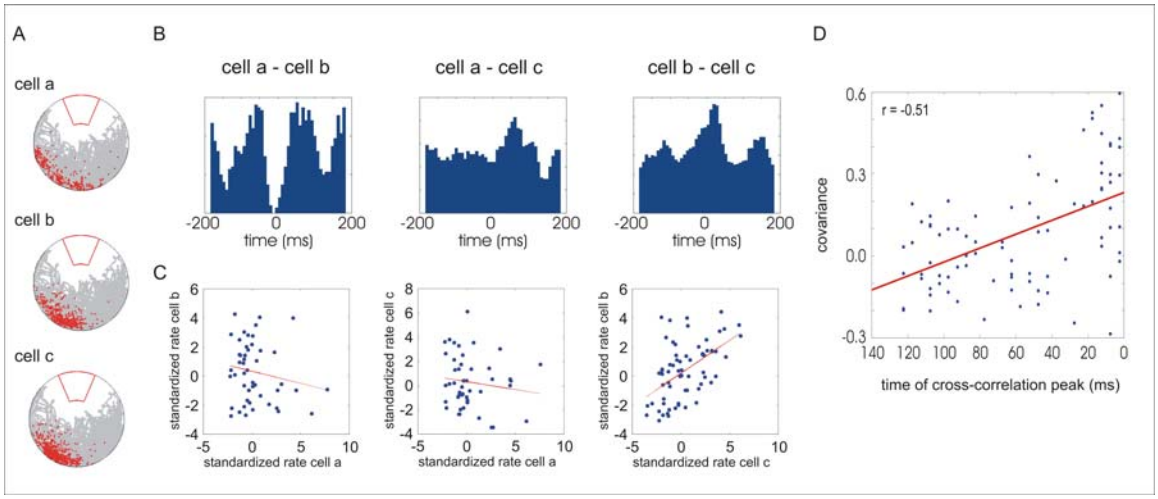

Figure 5. Comparing the temporal organization of firing on timescale of milliseconds and seconds. A) Spatial firing of three simultaneously recorded place cells with overlapping firing fields. B) Cross-correlation characterizes the coordination of firing of these cells on timescale of tens of milliseconds, C) The covariance characterizes firing of the same cell pairs during 5 second time intervals. D) The coordination of firing of a pair of cells on the two timescales is related. The cells that tend to fire closely together on the millisecond timescale tend to have high spatial-temporal covariance on the timescale of seconds.

We studied whether the spatial-temporal covariance is related to the similarity between representations coded for by the two cells. We observed that the covariance in the firing of neurons is positively related to the similarity between their firing fields ($r=0.48$, $p<0.001$, Fig. 4C1). The relationship between the covariance and firing field similarity was not present in the simulated data ($r=0.05$, $p=0.61$, Fig. 4C2), although simulation preserved the average firing rate at each location of the arena, so that the simulated firing rate maps looked the same as the real firing rate maps. This analysis indicates that relationship between covariance and firing field similarity is a result of temporal organization of cell discharge that cannot be directly predicted from the firing rate maps alone. It supports the notion that the cells' firing is organized in time on timescale of seconds.

Cells with similar average spatial responses tend to fire together, this was observed on the timescale of tens of millisecond and seconds. We studied the relationship between cells' correlations on these two timescales directly. Figure 5 shows that the time of cross-correlation peak is related to the covariance ($r=-0.51$); the better the firing of a pair of cells is coordinated on the theta timescale the better the firing is coordinated on the timescale of seconds. This relationship is particularly interesting because by itself the organization of activity on one timescale cannot explain the organization on the other timescale. The theta–scale organization of firing must be lost when activity is averaged over several seconds; and the organization observed in five-second intervals provides no prediction about the organization on the timescale of tens of milliseconds. Dargoi and Buzsáki (1996) made similar observation in rats running on a linear track.

Our analysis provides evidence that functionally-related groups of hippocampal cells are organized in time. We showed that the activity of cells is organized on different timescales and the temporal coordination of cell discharge is related to the similarity between response properties of the cells.

(Used programs: analyzeCovariance_list.m; CorrTwoFiles.m; ExpectedSpikesFromTS.m; MatchIntervals.m; crosscor.m; analyzeCC_list.m; AnCCst1.m; OrgCC2sess.m; CorrRATE.m; AnCCst1; AnMapst1)

3.5. Two states in the hippocampal ensemble activity during room+arena+ place avoidance

In this chapter we will show that ensemble activity during the room+arena+ place avoidance on a rotating arena is switching between two distinct states. Activity pattern during one of these states is similar to pattern during (room&arena)+ place avoidance on a stable arena.

In previous two chapters we saw evidence for groups of cells with similar functional responses to be active together. In this chapter we further investigate how hippocampal discharge is organized in time. We test the possibility that the hippocampal ensemble activity is changing between different states within a single session of the room+arena+ place avoidance task.

In chapter 3.3. we showed a tendency of simultaneously recorded cells to organize their firing in the same spatial reference frame, when two distinct frames were concurrently relevant. How to reconcile the tendency for unified ensemble responses with the need to encode two representations? Switching of the network activity between distinct network states could provide a potential solution to this problem. The hippocampal cells could at any moment respond in a unified way, and at different times of a session the network activity could alternate between different representations. In this chapter we explore the possibility that the hippocampal ensemble activity may be changing between different states within a single session of the room+arena+ place avoidance task.

Changes in hippocampal activity between different spatial or behavioral contexts have been observed and characterized previously. Hippocampal activity changes between different environments (Muller and Kubie, 1987; Lever et al., 2002; Wills et al., 2005; Leutgeb et al., 2005) as well as between different tasks within the same environment (Markus et al., 1995). Results of Fenton and Muller (1998) suggest that hippocampal place cell discharge is extremely variable even while a rat is performing a single task in a

single environment. Under such stationary conditions the ensemble activity may be changing between distinct states (Jackson and Redish, 2007; Lytton et al., 2007).

Two states in hippocampal activity during room+arena+ place avoidance

To analyze potential switching between different states of hippocampal activity, the network activity had to be characterized on a short timescale. First we chose 10-second intervals. During this interval the rat moved approximately 100 cm, so he could sample large proportion of the arena and visit firing fields of multiple place cells. Each 10-second interval was represented by an ensemble vector that contained the number of spikes emitted by each of the recorded cells (Chapter 2.5. provides more details about the methods). Pearson correlation between ensemble vectors was used to compare ensemble activity during different time intervals. Figure 1 shows the correlations of ensemble vectors during a single session of (room&arena)+ place avoidance on stable arena followed by a session of the room+arena+ place avoidance task on a rotating arena. The correlation during different intervals of the stable session is high (lower left hand part of figure 1B). During rotation time intervals with high correlation are alternating with time intervals when correlation is low, creating a checkerboard-like pattern (the upper right hand part of Fig. 1B). (Fig. 3A shows another example.) If the network activity pattern was switching between two or more states during rotation, a pattern like this could be expected.

Comparing the ensemble activity with stable conditions provides more evidence for switching between distinct activity patterns during rotation. The lower right-hand part (and symmetrical upper left-hand part) of the correlation matrix plot shows correlation of activity during the stable and the rotating conditions. This field of correlation matrix is dominated by blue color, signifying low or negative correlation between hippocampal activity during the two conditions. However, stripes of warm colors (some of them are marked by red arrowheads) in this predominantly blue area indicate that at certain times of the rotating session of room+arena+ place avoidance the ensemble activity was highly correlated with the activity during preceding (room&arena)+ place avoidance task on the stable arena. This suggests that one of the two activity patterns present during rotation is similar to activity on the stable arena.

The changes in the state of the ensemble activity with time are shown in figure 1C. The plot shows the average correlation of each 10-second interval with the stable conditions. When the arena is stable the average correlation with the stable session is high, as expected. When the arena is rotating the periods of high correlation alternate with periods of low correlation. The instances of high correlation with the stable-arena representation that were highlighted by red arrowheads in figure 1B are marked again.

Analysis of ensemble activity during one-second intervals

The same correlation analysis was performed using one-second intervals, and again it showed two distinct states of ensemble activity. For each one-second interval during arena rotation the average correlation with activity in stable condition was computed. In addition, for each interval during rotation the average correlation with other rotating intervals was also computed. When the average correlation with rotating condition is plotted against the average correlation with stable conditions (Fig. 2A) two clusters of points can be observed. One cluster corresponds to one-second intervals when correlation with rotating conditions was high and correlation with stable conditions was negative. The other cluster corresponds to intervals when the correlation with rotating conditions was lower and correlation with stable conditions was positive.

The time sequence of the switching between the two states is shown on figure 2B. The difference between normalized correlations with stable conditions and normalized correlation with rotating conditions was computed. The histogram of values of these correlations shows bimodal distribution confirming existence of the two states of the system (Fig. 2C).

The same analysis was performed in 12 recording sessions from six rats when at least 10 complex spike cells were active during stationary and/or rotating conditions. The analysis of correlation matrix indicated two distinct states in ensemble activity during rotating conditions in six of the 12 sessions from four different rats.

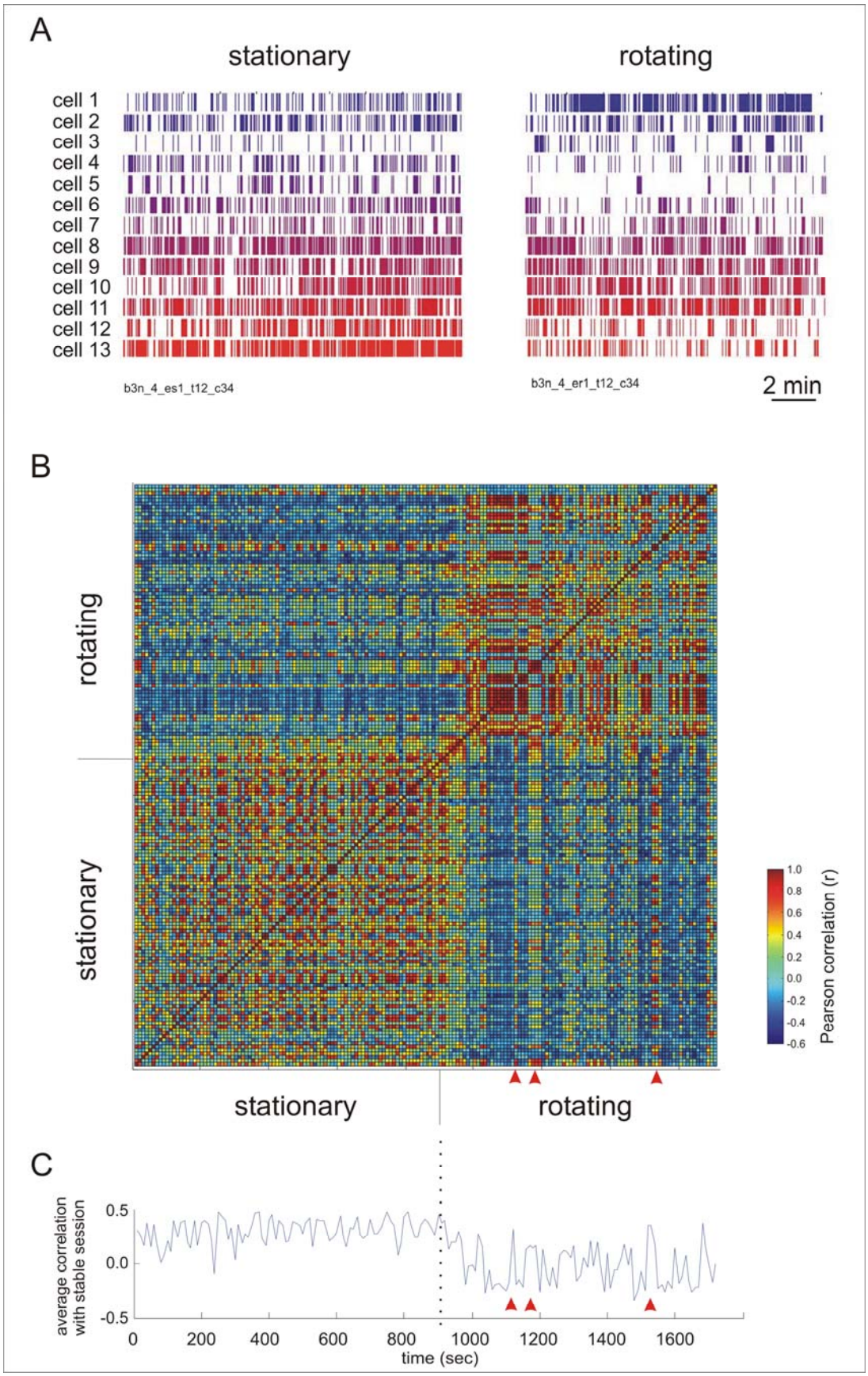
A
stationary
rotating
cell 1
cell 2
cell 3
cell 4
cell 5
cell 6
cell 7
cell 8
cell 9
cell 10
cell 11
cell 12
cell 13
b3n_4_es1_t12_c34
b3n_4_er1_t12_c34
2 min
B
rotating
stationary
stationary
rotating
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
Pearson correlation (r)
C
average correlation with stable session
0.5
0.0
-0.5
0
200
400
600
800
1000
1200
1400
1600
time (sec)

Figure 1. Change in hippocampal representation between stable and rotating arena in the same environment. A) Raster plots of an ensemble of 13 complex-spike cells that were recorded during a session of (room&arena)+ place avoidance task on a stable arena followed by a session of room+arena+ place avoidance on a rotating arena. A change in ensemble activity can be observed between the stable and rotating conditions. (this change in activity is similar to the change between two different environments shown in Fig. 1. of Chapter 2.5.) The ensemble activity during different 10-second intervals was compared. B) The correlation matrix shows correlation of neuronal activity for each pair of 10-second intervals. Different 10-second intervals recorded during the stationary conditions tend to have high correlation, as is indicated by the predominantly red color of the lower left-hand part of the correlation matrix. Similarly, the 10-second intervals recorded during the rotating conditions tend to have highly correlated activity, as is indicated by the predominantly red color of the upper right-hand part of the correlation matrix. Intervals during rotation are often dissimilar to the intervals during stationary conditions, as is indicated by blue pixels in the lower right-hand and upper left-hand part of the correlation matrix.
However, some 10-second intervals recorded during rotation are highly correlated with stationary conditions, as is indicated by reddish stripes marked by red arrowheads. These are intervals when the activity pattern resembled the pattern characteristic of the stationary arena.
C) Average correlation with stationary ensemble activity computed for each 10-second interval on stationary and rotating arena. The average correlation with the stationary ensemble vector is positive on stationary arena as expected, during arena rotation the value of average correlation fluctuates, it is negative at some times and positive at other times. The red arrowheads again mark some of the instances during rotation when correlation with the stationary session was high.

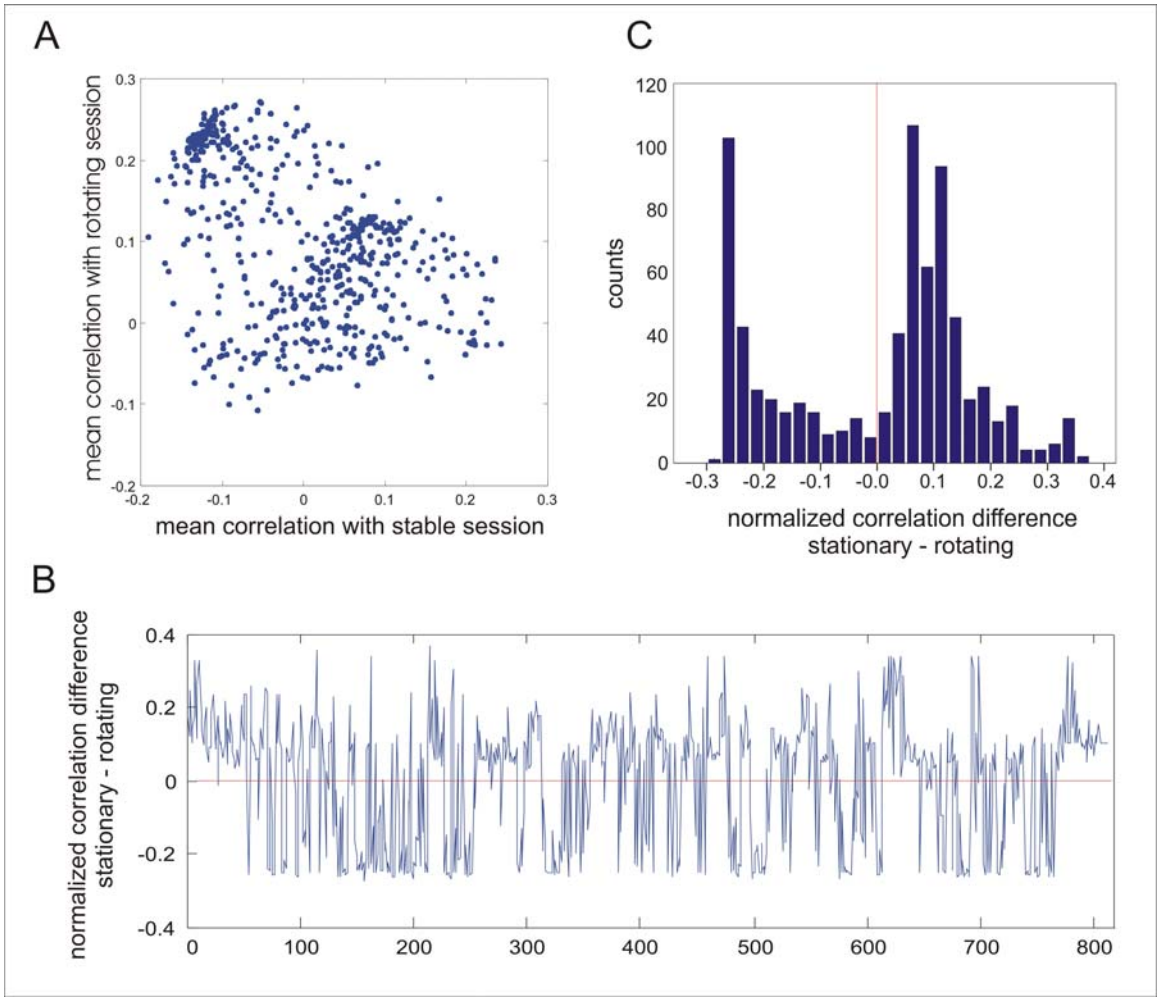

Figure 2. Two states in the ensemble activity during room+arena+ place avoidance on a rotating arena. The same ensemble as in figure 1 is analyzed. Only the rotating session data (but not the stationary session data) are shown here. A) For each one second interval on rotating arena correlation with stationary conditions is plotted against correlation with rotating conditions. Two clusters of dots, corresponding to two distinct states in the ensemble activity are apparent. One of the clusters has high correlation with activity on rotating arena, and negative correlation with activity on stationary arena. The other cluster has lower correlation with activity during rotation, and positive correlation with activity on the stationary arena. B) The temporal dynamics of switching between the two states. Correlation with stationary and rotating conditions was normalized and the difference is shown. The histogram of this normalized correlation difference is shown in plot C. The two states of ensemble activity can be clearly seen again.

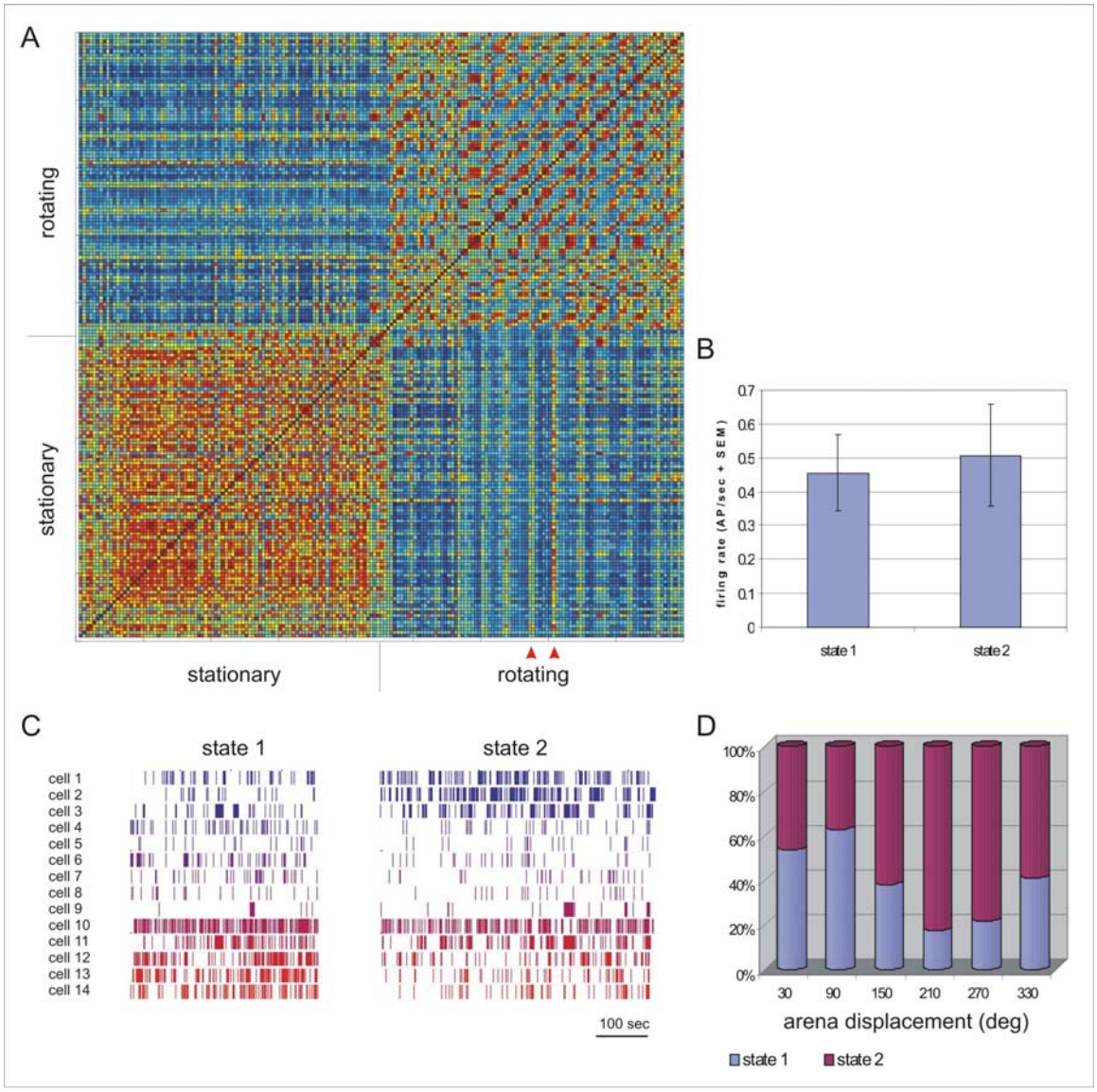

Figure 3. Correlation matrix showing activity of another hippocampal ensemble on stationary and rotating arena. As in figure 1B, some intervals recorded during rotation are highly correlated with stationary conditions (marked by red arrowheads). These are intervals when the activation pattern during rotation was similar to the pattern characteristic of stationary arena. Based on the similarity with stationary conditions, the ensemble activity during rotation was separated into two states. B) The overall mean firing rate of pyramidal cells was not significantly different between the two states on a rotating arena. C) The "raster plots" show distinct activity of some of the cells during the two states. For example, cell 2 was more active in state 2 than in state 1, while cell 12 was more active in state 1. D) The probability of one or the other state was different depending on the arena displacement in the room. The probability of activity being similar to stationary conditions (state 1) was higher when the arena displacement was similar to stationary conditions.

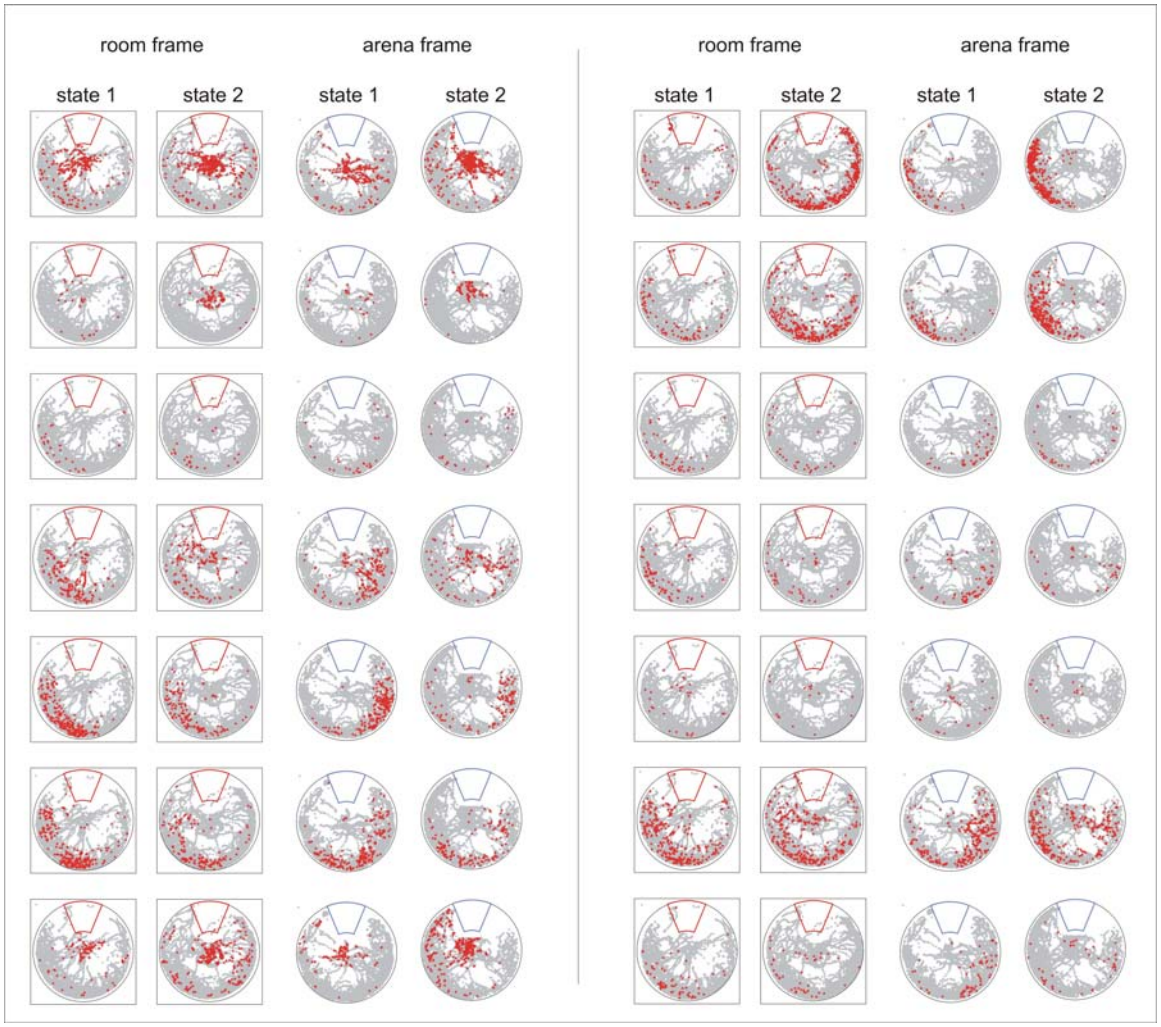

Figure 4. Spatial responses of 14 cells during two states identified during room+arena+ place avoidance on a rotating arena. Four spike maps were created for each cell; for each of the two spatial reference frames two maps representing two states were created.

Comparing the two states

Figure 3 shows another example of ensemble responses on stable and rotating arena. As in the previous example, remapping between stable and rotating conditions can be observed. On a rotating arena the ensemble activity during some 10-second intervals was highly correlated with activity on the stable arena. Based on the similarity with stable arena conditions two states could be detected in the ensemble activity during rotation. Although the overall firing rate was not different during the two states ($t=-0.28$, $df=26$, $p=0.78$; Fig. 3B), some of the cells discharged at different firing rate during the two states as can be seen from raster plots in figure 3C. Spatial distribution of firing of cells in the

two states confirms that some cells fire in one of the states, while other cells in both (Fig. 4).

The state of the ensemble activity was related to the displacement of the arena in the room (Fig. 3D). Once per each cycle of the arena rotation, the arena shock-zone was aligned with the room shock-zone; at that moment the arena was in the same position as during stationary session. When the arena position was most similar to the stationary session, the ensemble activity was most likely to be similar to activity on stationary arena.

These data show a change between two hippocampal states during room+arena+ place avoidance on a rotating arena. This is consistent with previous reports of existence of several distinct patterns of hippocampal activity within a single recording session (Jackson and Redish, 2007; Lytton et al., 2007). We also identified the representations corresponding to the two states. One of the states is unique to the rotating arena, while the other state is highly similar to activity on stationary arena.

(EnsembleVectorsFromTS.m; CorrMatAn.m; FindStates.m) To create raster plot of spikes during the two states use CorrMatAn.m to select the intervals in one and the other state. Then use Intervals.m to change the switch time from "line in .TS file" format to a "spike time in .TS file" fomat. Then use RasterPlotsSplitSession.m to generate the raster plots for the two states.

4.1. Discussion

Review of main findings

In our experiments the rats organized their spatial behavior in two distinct, concurrently relevant spatial reference frames. We showed that the rat's position in both reference frames was reflected in discharge of CA1 pyramidal neurons. Our main question was how the two reference frames are coordinated. We started by studying time-averaged data in firing rate maps and used information measures to assess frame preference within the entire recording sessions (chapter 3.3.). Then we analyzed temporal relationships between single-cell discharge on the time scale of the theta rhythm (chapter 3.4.). And finally we analyzed the ensemble activity on timescale of seconds (chapter 3.5.). Using this diverse range of analysis techniques we were able to detect a common motif in organization of hippocampal discharge. We consistently observed a tendency for groups of cells with a shared function (the same frame preference) to be organized together. Cells within a functionally defined group tended to be observed together within the same experimental sessions, and they tended to discharge together in time on timescale of physiologically important theta rhythm. Such grouping of cells with shared functional responses may help to coordinate – and prevent interference between – distinct concurrently relevant representations.

Let us summarize the main experimental findings:

The first aim was to study whether neurons recorded within the same session code for the two spatial frames independently, or whether cells that process the same frame are more likely to be recorded together. We observed a significant tendency for the cells recorded in the same session to encode the same reference frame. Simultaneously recorded neurons were not coding the two reference frames independently.

The second specific aim was to study whether different representations are organized in time on the scale of the theta rhythm. We observed temporal organization of discharge on this physiologically important timescale. As reported previously, the temporal organization was related to the similarity in the response properties of the cells.

On stable arena the cells firing closer in time had more similar spatial firing than cells firing at different times. Extending previous reports, we found that temporal organization in activity of cells may play a role in coordinating the two reference frames. In the room+arena+ place avoidance task the cells coding for the same reference frame were more likely to discharge within 20-30 ms from each other. These observations are consistent with the hypothesis that temporal binding of activity of cells with shared function is a mechanism used to organize distinct, simultaneously relevant representations.

The third specific aim was to determine whether the hippocampal representation is stationary throughout the experimental session or whether there is an indication of switching between different network states. We observed different hippocampal activity patterns in stationary and rotating conditions in the same physical environment, in accord with previous reports of different representations being activated in different behavioral contexts (Markus et al., 1995). During the rotation there were episodes of time when the hippocampal activity was highly correlated with stationary conditions, and periods when the activity was distinct. Switching of hippocampal activity between two states on the timescale of seconds can provide yet another mechanism for coordinating distinct representations.

Cohesiveness in CA1 responses

A series of experiments from different laboratories were performed to study whether the responses of CA1 neurons are cohesive, or the cells are acting independently (Tanila et al., 1997; Brown and Skaggs, 2002; Knierim, 2002; Fenton et al., 2000; Lee et al., 2004). These so-called double rotation experiments shared similar design; place cells were recorded in two sessions. Between the first and the second session some of the landmarks were rotated in a clockwise direction and other landmarks in a counter-clockwise direction. Responses of ensembles of place cells during the second session (a probe session) were studied. Did all the cells respond cohesively following the same set of landmarks, or did they behave independently some following one set of landmarks, and some the other? These experiments did not lead to conclusive results; both cohesive responses and independently acting units were reported in CA1. Two shortcomings could

have contributed to the ambiguous results of these studies. First, cell responses changed between the two sessions in a way similar to remapping. Lee et al. (2004) reported that only 21.7% of CA1 cells with one firing field in the first session remained firing field in the second session, vast majority of cells gained or lost their firing fields between the two sessions as the landmarks were rotated. Similarly Shapiro et al. (1997) observed a tendency of independent representations to develop between the first and the second session. This suggests that the cells did not follow one or the other set of cues, but hippocampal representation changed completely during the probe session, making the results hard to interpret. It would be optimal to study the cohesiveness of CA1 responses in a system that is not changing. Room+arena+ place avoidance task provides such a paradigm where cohesiveness of responses can be studied in a steady state conditions, without a probe trial, within a single experimental session.

The second potential shortcoming of double rotation experiments comes from the fact that the responses during the sessions were averaged, preventing analysis of organization within a session. If activity of cells was organized in shorter timescale, it could have been unnoticed as a result of averaging. In our analysis we not only studied time-averaged responses, but also studied dynamics of hippocampal activity on short time scales up to tens of milliseconds.

Our experiment differed from the double rotation experiments in a third potentially important way. In our paradigm the rats were reinforced to organize their behavior in the two spatial frames, while in previous experiments there was no reinforcement for processing the two sets of landmarks.

We observed a tendency for cohesive responses among CA1 pyramidal cells – neurons within the same recording session were predominantly responding to the same spatial frame. In CA3 even stronger tendency for grouping can be predicted. Previously, more concordant responses were observed in CA3 compared to CA1 (Lee et al., 2004; Leutgeb et al., 2004; Vazdarjanova and Guzowski, 2004).

Anatomical functional grouping in the hippocampus?

Anatomical grouping of neurons according to their function is a fundamental organizational motif of vertebrate sensory and motor areas (Mountcastle, 1957; Hubel

and Wiesel, 1962). Neurons with similar response properties are anatomically grouped together forming barrels in the somatosensory cortex (Woolsey and Van der Loos, 1970), or cortical columns in the visual areas (Hubel and Wiesel, 1962). In the cortical sensory and motor areas neuronal responses are largely hard-wired early in development, the neurons respond the same way across different behavioral contexts. If the same cortical network is always together processing the same or similar information, then it is economical to have those neurons anatomically close to each other. In the hippocampus, neuronal responses differ in different spatial and behavioral contexts (Muller and Kubie, 1987; Markus et al., 1995; Lever et al., 2002; Wills et al., 2005; Leutgeb et al., 2005). Hippocampus receives polymodal, pre-processed information and depending on the context it responds by creating potentially arbitrary associations between inputs. Theoretical work suggests that if a network processes large numbers of arbitrary associations the neighboring neurons should have uncorrelated inputs and response properties in order to preserve flexibility and to increase storage capacity of the network (Marr, 1969).

Anatomical functional grouping has only rarely been observed in polymodal association areas. In primates, neighboring perirhinal cells were more likely to respond to familiar pictures, suggesting familiarity induced a functional grouping (Erickson et al., 2000). Question of anatomical functional grouping in the hippocampus is a matter of controversy (Hampson et al., 2002). In rat hippocampus, the firing fields of place cells within ~1 mm were reportedly closer than expected by chance (Eichenbaum et al., 1989). In the delayed non-match to sample task Hampson et al. (1999) observed that cells within 600 – 800 μm had similar responses. Cells were grouped according to their responses to the left, or right lever-presses, or according to the phase of the task. However, a thorough study of over 3000 place cells failed to reveal any similarity between firing fields of anatomically adjacent cells on a linear track (Redish et al., 2001).

Our results may contribute to this discussion. The vast majority of cells we recorded were not further than 600 μm from each other. We saw the same frame preference in cells within this distance, but we cannot answer the question whether the regions further apart had different or same frame preferences. We can conclude, however, that the frame preference within the same region could change between sessions. This is different from

reports of Hampson et al. (1999) who saw the same response properties of neurons in the same anatomical locations even between different rats.

Attractor network hypothesis, maplets, and cell assemblies

How do our results relate to an attractor network model (Samsonovich and McNaughton, 1997; Tsodyks, 1999), cell assembly hypothesis (Harris et al., 2003) or maplet model (Touretzky and Muller, 2006) of hippocampal network function? Our observation that neurons were not acting independently is consistent with these models. However, not all of simultaneously recorded neurons had the same frame preference. Observation of pairs of cells with discordant frame preferences is consistent with the notion of cell assemblies or maplets. It is not consistent with the attractor model in its purest form that would suggest that the same attractor, corresponding to a single reference frame, is active during the entire experimental session. Our observation that the network can be switching between different states within a single session is relevant in this context. This would allow different attractors to be active at different times. If switching between states was occurring within a session, the strong attractor properties, even if they were present, could not be observed in data averaged over the entire session.

Temporal organization of representations on timescale of tens of milliseconds

The idea that cells participating in the same representation should discharge together in time was suggested as a solution to the binding problem, or superposition catastrophe (Milner, 1974; von der Malsburg, 1981, 1999; Gray, 1999). Our evidence for grouping of neuronal discharge according to spatial frame is consistent with this hypothesis. On stationary arena we showed that cells firing close together within a theta rhythm are coding for more similar locations than cells active separately in time. Organization of hippocampal activity on timescale of theta rhythm is not unexpected: theta organizes hippocampal discharge (O'Keefe and Recce, 1993), timing of spikes relative to a phase of theta provides information about the position of the rat in an environment (Huxter et al., 2003) and helps to organize the activity of neurons into temporal sequences (Dragoi and Buzsáki, 2006). Theta frequency stimulation is an efficient protocol to induce long-term potentiation in the hippocampus (Larson et al.,

1986), suggesting that neuronal activity organized at theta rhythm timescale may be involved in organizing synaptic plasticity under physiological conditions (Pavlides et al., 1988; Otto et al., 1991; Hölscher et al., 1997; Hyman et al., 2003).

In the room+arena+ place avoidance task the cells coding for the same spatial reference frames (concordant cell pairs) had a significant tendency to fire within 20-30 ms. This tendency was not observed in discordant cell pairs (coding for different spatial reference frames). The preferred timing within 20-30 ms matches membrane time constant of pyramidal neurons (Spruston and Johnston, 1992) and period of hippocampal gamma oscillations (Csicsvari et al., 2003). The organization of neuronal firing in the hippocampus on this timescale was reported before (Harris et al., 2003). The authors speculated that cells firing characteristically within this time window are part of the same cell assembly. Our data suggest that when two representations are relevant concurrently, such temporal coordination mechanism can be used to organize the groups of cells participating in representation of the same reference frame.

Temporal organization of representations on the timescale of seconds

Our analysis provides evidence for two distinct alternating states in the population activity within a session of the room+arena+ place avoidance task. One state correlated with the activity in stationary conditions, the other state was distinct. Indications of switching of the hippocampal activity without apparent changes in the rat's task have been reported previously. Fenton and Muller (1998) reported overdispersion in the single cell firing within an experimental session. They showed excessive, unexplained variability in the hippocampal discharge within stationary experimental conditions. Theoretical work of Olypher et al. (2002) suggested that changes in hippocampal "state" on the timescale of few seconds may be underlying this excess variability in the cell's firing. The changes in the hippocampal activity were observed on the level of simultaneously recorded ensembles of cells (Lytton et al., 2007; Jackson and Redish, 2007). All these data point to a possibility of the hippocampus "switching" between at least two distinct states. In our experiment we also observed switching between two states, and could associate the states with two different representations for two different conditions. Such switching provides evidence that organization of neurons into

functionally related groups may be the mechanism for coordinating different representations.

Possible clinical implications

We worked with the hypothesis that the coordination of activity of neurons into functionally related groups underlies the ability to process and organize distinct neural representations (mental objects, thoughts, perceptions). We can then speculate what the consequences of impaired neural coordination would be. Inappropriate associations between stimuli could be manifested as disorganized thoughts such as observed in psychosis.

Hippocampal pathology has been implicated in schizophrenia and psychosis. Some studies reported decrease in volume of hippocampus and related structures in medial temporal lobe in schizophrenia (Sim et al., 2006). Shenton et al. (2001) reviewed magnetic resonance imaging (MRI) studies and concluded that schizophrenia is associated with abnormalities in medial temporal lobe structures, including hippocampus, together with ventricular enlargement, changes in frontal (particularly prefrontal cortex) and parietal cortex, basal ganglia, and corpus callosum. Although the changes in hippocampus are among the most prominent, they are rather subtle. Meta-analytic study of Nelson et al. (1998) reported reduction of hippocampal volume by 4 % in schizophrenia, and number of studies did not detect significant change. For example, an epidemiologically representative study of Tanskanen et al. (2005) showed small reductions in hippocampal volume in schizophrenia (2 %) and all psychoses (3 %), which was insignificant when adjusted for the total brain volume reduction.

The lack of dramatic anatomical changes in schizophrenia suggests that perhaps functional changes may underlie the pathology. Abnormalities in neural synchronization have been associated with certain brain disorders like epilepsy, schizophrenia, autism, Alzheimer's disease and Parkinson's disease (Uhlhaas and Singer, 2006). Uhlhaas et al. (2006) studied neuronal synchronization in patients with schizophrenia using scalp electroencephalography. They reported decrease in long-range synchrony in beta frequency range (20-30 Hz) in patients with schizophrenia compared to control subjects. Kao and Fenton (2007) studied neural synchrony in hippocampus in a rat model of PCP

induced psychosis. They observed increased synchronization in hippocampal single cell activity on the timescale of hundreds of milliseconds. In parallel, they tested the rat's ability to organize relevant and irrelevant information in a set of behavioral tasks. The PCP impaired rats' behavior in tasks with high demands on ability to segregate relevant and irrelevant information, but PCP had no effect when the demand for cognitive segregation was low. Interestingly, the effect of PCP on neuronal synchrony was only observed in dose (5 mg/kg) that induced behavioral impairments. Lower dose of PCP (3 mg/kg) did not induce detectable changes in neither rat behavior, nor the cell synchronization. These results suggest that temporal organization of hippocampal discharge may be a mechanism underlying hippocampal information processing in the physiological as well as pathological conditions.

Hippocampus was also implicated in mood disorders. Smaller hippocampal volume was reported in people with post-traumatic stress disorder (Bremner, 2002) or major depression (Sheline et al., 1996). Moreover, Gilbertson et al. (2002) who studied monozygotic twins with different traumatic (military combat in Vietnam) experiences, found that the smaller hippocampus is a risk factor, rather than consequence of the pathology. Similarly in animals, hippocampal function and structure is affected by chronic stress (Magariños et al., 1997), but at the same time there is also evidence that hippocampal size predicts the behavioral outcome of stress (Lyons t al., 2001). This shows that besides being sensitive to stress, hippocampus plays a role in regulation of the stress response.

The tendency to overgeneralize responses to an inappropriate context is characteristic for human patients with affective disorders (Carver and Ganellen, 1983; Ganellen, 1988). Overgeneralization is also observed in animal models of affective disorders. For example, serotonin 1A receptor (5HT1A) knockout mice show enhanced fear conditioning to ambiguous conditioned stimuli (Tsetsenis et al., 2007). In ambiguous environments containing a mixture of familiar threatening landmarks as well as novel neutral landmarks, the 5HT1A knockout mice have a stronger tendency for a fear response than wild type mice (Klemenhagen et al., 2006).

The hippocampus plays a key role in contextual learning (Anagnostaras et al., 2001; Smith and Mizumori, 2006). Hippocampal place cell activity is specific for different spatial (O'Keefe and Dostrovsky, 1971; Muller et al., 1987) as well as behavioral contexts (Markus et al., 1995). The impairment in the ability to distinguish contexts and to switch between them when appropriate could lead to generalization of thoughts and behaviors across different, perhaps inappropriate, contexts.

The serotonergic system has been implicated in mood disorders, and antidepressant and anxiolytic drugs often enhance serotonergic neurotransmission. Could serotonin have an effect on hippocampal ability to encode differences between contexts? The 5-HT1A receptor modulates hippocampal synaptic plasticity, and serotonin is more important for plasticity in novel environments than in familiar environments (Sanberg et al., 2007). Serotonin increases inhibition at the somata and proximal dendrites of CA1 pyramidal cells and therefore suppresses the intra-hippocampal synaptic communication (Gulyas et al., 1999). On the other hand, serotonin acting via 5HT1A receptors decreases inhibition at distal dendrites and therefore potentially enhances the neocortical input to hippocampus (Gulyas et al., 1999). Higher sensitivity to neocortical input could enhance processing of environmental information, and therefore improve novelty detection. On the other hand, decreased serotonin levels could lead to impaired recognition of environmental landmarks, and consequently to overgeneralization of representations across different contexts.

This thesis explored potential mechanisms of coordinating distinct representations. We found that cells sharing the same frame preference have activity grouped together, which provides a way to avoid interference between distinct representations. Such grouping of activity of cells with shared function was observed on timescale of entire sessions as well as on timescale of the theta rhythm. Grouping was observed in activity of cell pairs as well as on the level of hippocampal neuronal ensembles. Understanding mechanisms of coordination of different representations is not only of theoretical importance, but may have implications for studying still perplexing disorders of mind.

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