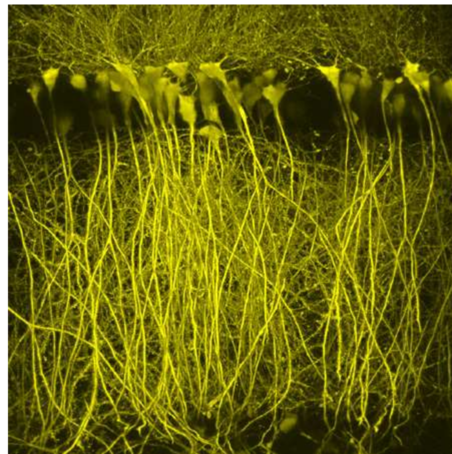
# An electrophysiological study in freely exploring rats

## - Place cell properties and place field stability in the absence or presence of reward as a motivational factor

by

Susanne Søgnen



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# Summary

The activity of pyramidal cells in the hippocampus can be spatially modulated. Numerous studies have been performed examining their properties, like what influences the position of their firing fields or what effect changes in the environment have. Most of these studies used reward to motivate the rat, keeping its activity level high, which is important for optimal recordings.

In order to record single cell activity male Long Evans rats were implanted with four tetrodes, totalling 16 electrodes, in the CA1- region of the hippocampus. Three testing apparatuses were used. The first, a circular one, was used while we were searching for complex spike activity. The next two were square and identical except for the content, one was empty but the rat received a reward during the sessions, the other contained four objects and the rat did not receive a reward. Our aim was to compare the development and stability of the place fields in the presence and absence of reward.

Place cell activity was only found in two of the rats implanted. One of these rats was tested in all three of the enclosures, and manipulations were made to the cues. For the other rat recordings were made in the circular apparatus and in the one containing objects, no cue manipulations were made.

We found stable place fields in the presence and absence of reward. The firing fields appeared to be better defined when objects were present than in the empty enclosure where reward was given.

The presence of objects in a testing enclosure affected the behaviour of the subjects. The activity level was just as high in reward and non-reward conditions, however the behaviour pattern differed.

Rotating the cue card placed on the wall of the enclosure showed that other cues than this card were available to the rat.

Due to our limited amount of data it is difficult to draw any conclusions. It is however clear that with the conditions used in this study stable place fields developed in the presence and absence of reward.

# Samandrag

Aktiviteten til pyramidalceller i hippocampus kan vere modulert av spatiale faktorar. Fleirfaldige studiar er gjennomført der ein studerer eigenskapane deira, som til dømes kva som påverkar plasseringa av fyringsfelta og effekten av endringar i omgjevnadane. Dei fleste av studiane brukar påskjønning for å motivere rottene og dermed halde aktiviteten høg og få optimale registreringar.
For å registera enkeltcelleaktivitet vart fire tetrodar, 16 elektrodar, implantert i CA1-området i hippocampus på Long Evans- hannrotter.
Tre testapparat vart brukt i denne studien. Eit rundt der testsesjonane føregjekk så lenge vi leita etter ”complex spike” aktivitet og to kvadrat. Desse to var identiske med unntak av innhaldet, då den eine var tom og rottene mottok påskjønning undervegs i sesjonane, i den andre var det plassert fire objekt, men inga påskjønning vart gitt. Målet var å samanlikne utviklinga og stabiliteten til stadfelt med og utan påskjønning.
Det vart berre funne stadcelleaktivitet hos to av dei implanterte rottene. Hos den eine av desse vart det registrert sesjonar i alle tre apparata, og manipulasjonar vart gjort med dei tilgjengelege landemerka. Hos den andre rotta vart det registrert sesjonar i det runde apparatet og i det som inneheldt objekt. Det vart ikkje gjort nokon manipulasjonar.
Hos begge rottene vart det funne stabile fyringsfelt både med og utan bruk av påskjønning. Fyringsfelta såg ut til å vere tydelegare avgrensa når det var objekt til stades enn når påskjønning vart gitt.
Objekt plassert i apparatet hadde ein tydeleg effekt på oppførselen til rottene. Aktivitetsnivået var om lag like høgt både i apparatet med påskjønning og i det som inneheldt objekt, men åtferdsmønsteret endra seg.
Ved å rotera arket som var plassert på innsida av den eine veggen i apparatet såg vi at andre landemerke enn arket var tilgjengeleg for rottene.
Grunna den avgrensa datamengda vår er det vanskeleg å trekke nokon konklusjonar av resultata våre. Hovudfunnet er at med dei føresetnadane som var i denne studien så utvikla det seg stabile fyringsfelt både med og utan påskjønning.

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And finally to my family and friends who have been patient with me. For your support and encouragement, thank you so much.

Bergen, May 2009

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Susanne Søgnen

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# Introduction

For all animals the ability to navigate in an environment is extremely important. The degree of complexity needed depends on where the animal lives, what abilities they have and how much they are depending on migration during their lives, but for any one of them navigation is crucial for survival. Navigation is important for the animal when searching for food or water, searching for mates, avoiding dangerous areas and finally finding its way back home after it has done what it set out to do. Humans have developed methods of navigation other than the ones that are part of our biology, like maps, compasses, GPS and so forth. These are invaluable tools when sailing across the ocean, when you are walking in the mountains, or when trying to find a place you have never been before. Still none of these aids are as impressive as the navigational system of the brain.
For humans, and any other animal, the brain takes the sensory information of prominent features of the surroundings, like sounds and smells, and transforms it into a multisensory representation of the environment.
For mammals the part of the brain responsible for making this representation is the hippocampus and its surrounding structures.

## *Functions of the hippocampus and studies thereof*

The hippocampus is a structure in the brain which is linked to the formation of memory, both spatial and non-spatial; it is also believed to participate in the linking of non spatial memories, like episodic memories, to the time and place where the remembered event occurred.
The hippocampus does not store the memories per se, it stores the pattern of activity that arose within other cortical areas when the event occurred, and thereby functions as an index to the memories stored in the brain. It causes the same areas of the brain to be reactivated if something happens that is similar to a previous episode.
Different parts of the hippocampus have slightly different tasks, for instance there is evidence that the ventral hippocampus is somewhat less important for the formation of spatial memories, lesions of the ventral hippocampus are less damaging to spatial memory than lesions in the dorsal part [2].

In 1948 E.C. Tolman proposed the theory of a cognitive map based on behavioural studies of rats. He proposed that humans also develop and use such a map. The theory was strengthened by John O'Keefe and Lynn Nadel when they in 1978 published "The Hippocampus as a cognitive map". The book postulated the hippocampal formation as the neuronal basis of the cognitive map.

In between these two events the hippocampus and the surrounding cortical areas became recognised as crucial for the formation of memories among others by Scoville and Milner in 1957, when they described their findings from intelligence and memory tests performed on several patients who had undergone extensive neurosurgery. In particular the results from a patient called H.M. were of interest. He had had the medial temporal lobe area removed bilaterally to relieve his symptoms of epilepsy. They found that he was not able to form new memories of any events following surgery and only able to recollect memories of events happening prior to 19 months before. They concluded that the hippocampus and surrounding structures had to be responsible for the formation of new memories [3]. H.M. was impaired in spatial memory tasks and in tasks involving declarative memory, memory that can be verbal (episodic and semantic memory). He was not impaired when it came to procedural memory, memory of skills and procedures, but he was unable to remember being taught the task.

That an intact hippocampus is necessary for learning to solve spatial navigation tasks have been shown in numerous studies (among others O'Keefe and Nadel, 1978 [4] and Jarrard, 1983 [5]), many other studies have shown that the firing of the hippocampal neurons relates to the position of the animal in space (among many others O'Keefe and Dostrovsky in 1971 [6], Muller et al. in 1987 [7], Wiener et al. 1989 [8]). These studies were all performed using rats; however the study including H. M. shows that the findings seem to apply to humans as well. This has later been confirmed using modern techniques, like PET or fMRI. Maguire et al asked taxi drivers to describe the routes they would take to get around the streets of London. While describing the route they would take the drivers underwent a PET scan. The scan showed a significant increase in activity in several brain areas, including the right hippocampus. In contrast to the other activated brain areas the hippocampus was not active when the subject was visualizing famous landmarks he/she did not know the location of. This indicates a specific activation of the right hippocampus in navigating a complex environment [9].

The importance of the hippocampus in humans in memory generation and consolidation becomes apparent in patients with Alzheimer's disease. In this neurodegenerative disease the

neurons of the hippocampus and the surrounding cortical areas are among the first to become affected.
The first symptoms of this disease are memory deficits, mainly in recollecting newly learnt facts, and disorientation. The memory deficits will, during the progression of the disease become more pronounced and often cause inability to learn new facts. However, older memories might be unaffected. This is explained by the hippocampus' involvement in creating new memories, but not necessarily in recollecting old ones.

By now the fact that the hippocampus and surrounding structures are involved in memory formation and also recollection is no longer a subject of debate, however what specific areas are responsible for the different aspects of memory and what mechanisms are involved is still not clear.
The hippocampus helps bind together the perceptions of the surrounding world provided by our senses. It is the joint impressions from all our senses that give us our perception of the world.

### *The anatomy of the rat hippocampus*

The rat hippocampus is a part of the hippocampal formation, which includes the hippocampus, the dentate gyrus, the subiculum, presubiculum, parasubiculum and the entorhinal cortex [10].
The anterior hippocampus is positioned right next to the septal nuclei in the basal forebrain, and it continues backwards in the shape of a C, extending over and behind the diencephalon [11]. The dentate gyrus is also C-shaped, and the opening of this C surrounds the lower tip of the hippocampal cortex, the CA4.
The rat hippocampus is quite large in proportion to the rest of the brain and it is a large part of the forebrain. As can be seen in figure 1 it looks kind of like two bananas, joined at the stem.

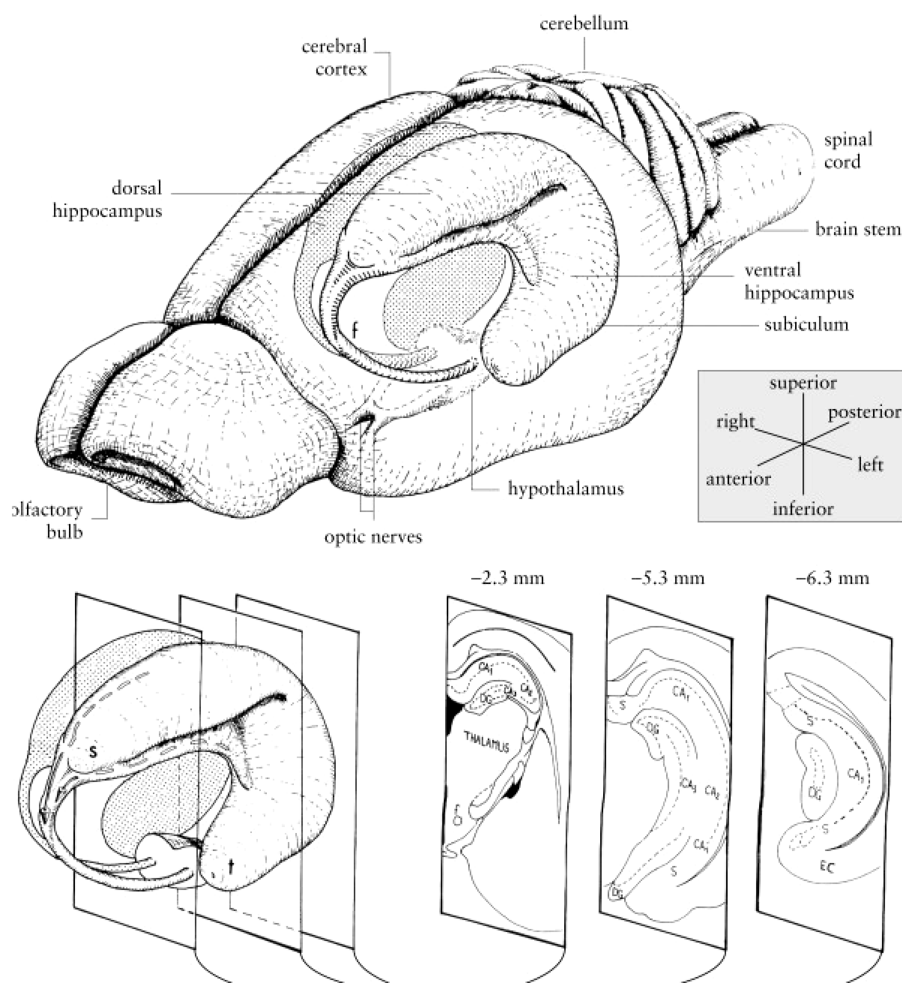



**Figure 1. The hippocampus and its three dimensional position in the rat brain**. Bottom half; three coronal sections showing the organisation of the cell layers at different distances to bregma. (Modified from *The rat nervous system 2nd ed.* [12]).

The hippocampus proper was subdivided by Raphael Lorente de No into four distinct regions, CA1, CA2 CA3 and CA4, with CA standing for Cornu ammonis, which means the horn of Ammon. These regions are distinct from one another in several ways, for instance in cell body size; the cell bodies become larger as one goes from CA1 to CA3. There is also a difference in the size and distribution pattern of the cells' apical dendrites [13]. Synaptic connections also differ, for instance CA3 receives input via mossy fibers from dentate gyrus, whereas CA1 does not [11].

The area called CA4 consists of the scattered cells in the hilus, the hilus being the area surrounded by the blades of the dentate gyrus. CA3 is the continuation of CA4, it starts where the blades of the dentate gyrus end and ends in the narrowing of the cell layers (see Figure 2).

This narrowing is designated CA2, a small area that connects CA3 to CA1 [14]. The end of the CA1 cell layer marks the beginning of the subiculum.

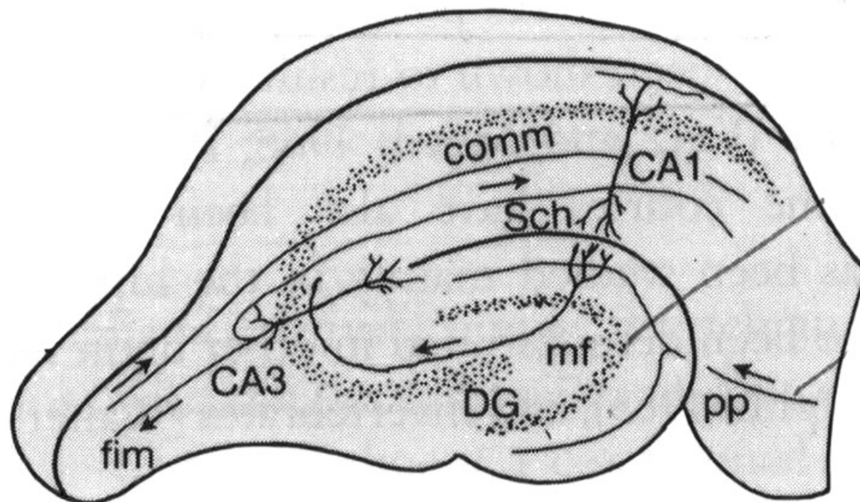



**Figure 2. A drawing of the cell layers of the hippocampus, the most prominent projections within it and the most important afferents and efferents.** Abbreviations: DG- dentate gyrus, mf- mossy fibres, comm.- commissural fibres, Sch- Schaffer's collaterals, pp- perforant path and fim- fimbria fornix.
From http://www.graulab.tamu.edu/J-Grau/Psyc340/Outlines/ComplexStim-Neurobio.html .

## The cell types in the hippocampus

In contrast to the multiple cell types of the neocortex the hippocampus proper consist of one type of principal cell, and several kinds of interneurons [14].

The principal neurons in the hippocampus are the pyramidal cells. These cells have a pyramidal shape with apical and basal dendrites (see Figure 3). Depending on which region of the hippocampus the cell is located in the size of the cell body varies from 20-40 µm at the base and 40-60 µm in height. The apical dendrite is quite long and can be seen quite easily under the microscope, it is 5- 10 µm at the beginning and gives off side branches. The dendrite can reach lengths of 500-1000 µm in the CA1- area, slightly shorter in CA3 [13]. The axon of the pyramidal cell originates in the basal part of the soma, and can be difficult to distinguish from the basal dendrites. An important property of the pyramidal cells of the hippocampus is that in any given environment only a fraction of them are active [15]. In other words the majority of them are silent at any given time.

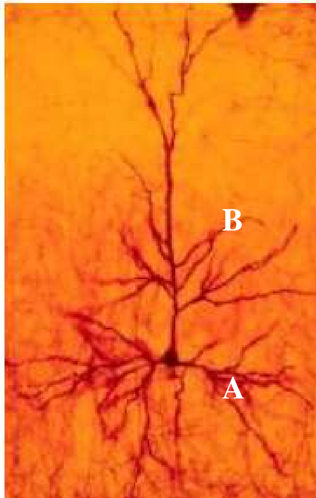



**Figure 3. A pyramidal cell located in the CA1-area.** Golgi stained. The A indicates the position of the cell soma. The apical dendrite is marked by the B. The basal dendrites can also be seen. Modified from http://www.neurostructural.org/home.asp ([1]).

Interneurons exist in all parts of the hippocampus; however they might differ depending on what region and which cell layer they are located in. For instance some interneurons are located close to the pyramidal cell layer of the hippocampus (and along the deep surface of the granule cell layer of the dentate gyrus), these cells are called basket cells [10]. Interneurons might differ in among other things size, dendritic length or dendritic organization. The vast majority uses GABA as the primary neurotransmitter [11].
A large number of the interneurons are so called theta cells (see *Theta cells*).

## Circuitry and connectivity

The connections between the hippocampal formation and the entorhinal cortex have been described as a trisynaptic loop by Andersen et al 1971. They described it as consisting of entorhinal cortex, dentate gyrus, CA3 and CA1 [16]. The main flow of information is from entorhinal cortex through the dentate gyrus to the hippocampus.

$$Entorhinal\ \ cortex \xrightarrow{Perforant\ \ path} dentate\ \ gyrus \xrightarrow{Mossy\ \ fibres} CA3 \xrightarrow{Schaffer\ \ collaterals} CA1$$

**Figure 4. The trisynaptic loop.** The main flow of information is indicated by the arrows.

Later studies have shown associational fibres within the respective CA-area, especially in CA3. There are synaptic connections in the opposite direction of the trisynaptic loop, for instance CA1 neurons project back to cells in the entorhinal cortex, and the entorhinal cortex projects directly to CA3 and CA1 as well as the dentate gyrus [10, 11].

There are also some commissural fibres projecting to the contra lateral hippocampal formation. These fibres, like the associational connections, mainly exist in CA3. The amount of commissural fibres varies from species to species [11].
All of these facts emphasises that the trisynaptic loop is a simplification of the complex information flow. Nevertheless is still a useful concept for understanding how inputs are distributed in the hippocampal formation [17].
A more detailed view on projections targeting the hippocampus was proposed by Lavenex and Amaral in 2000 [18].

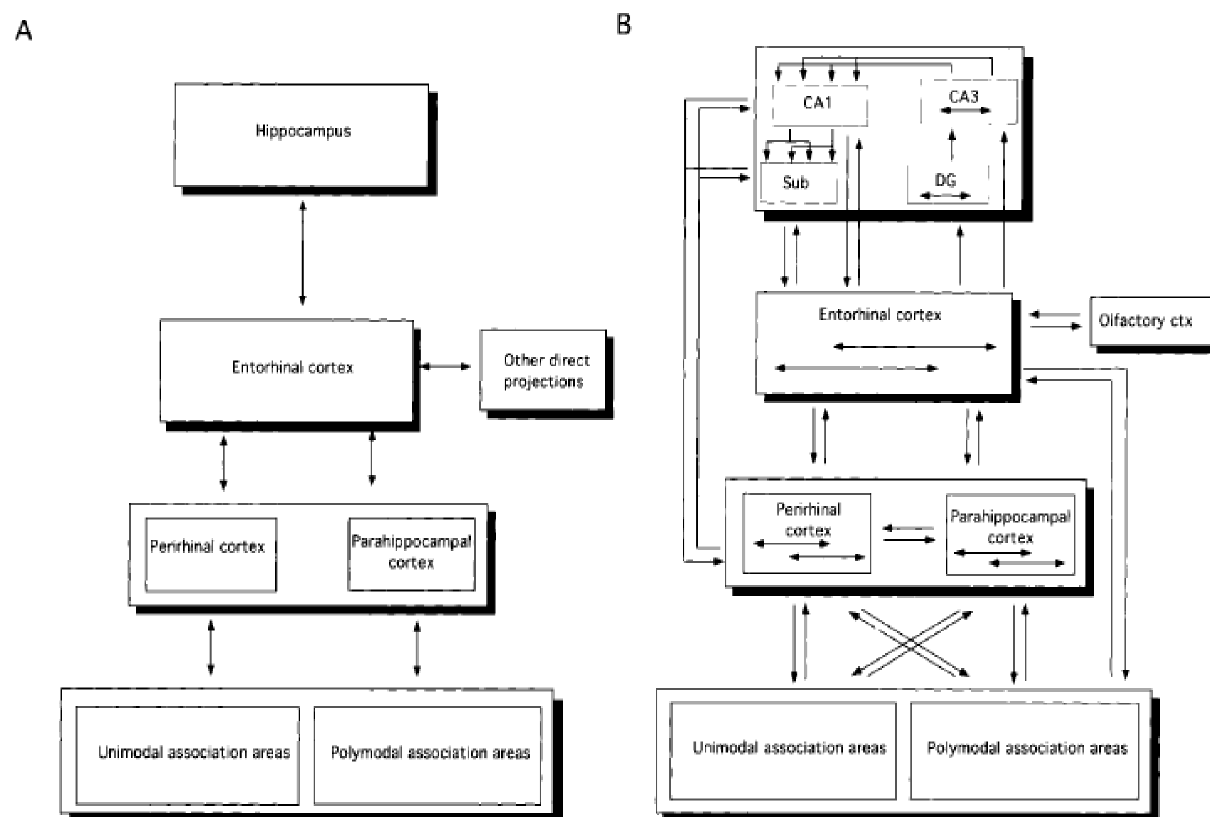



**Figure 5. A schematic presentation of the hierarchical order of the projections within the medial temporal lobe.** A. A simple presentation of the projections from the cortex to the hippocampus. B. A more detailed diagram which in addition to the hierarchical order also shows the associational connections on every level of the hierarchy. From Lavenex and Amaral 2000 [18].

They defined the cortices into a hierarchy where the complexity of the information increases when moving from the neocortex to the hippocampus.

The hippocampal formation receives direct input from a limited number of neocortical areas; many of these areas are convergence sites for afferents from a large number of other neocortical regions. The information received by the hippocampus is very different from the sensory information received by these neocortical areas. This is due to the information being processed on its way from the neocortex to the hippocampus. The transitional areas that convey information from the neocortex to the hippocampus probably do not do this passively, but participate actively in the information processing. Intrinsic associational connections on every level of the information transfer from the neocortex to the hippocampus are an important part of this information processing [18].

A good example of the processing of information within a hierarchical level is the afferents to CA3. The CA3 receives the majority of its input from two sources, the perforant path and the mossy fibres, originating in the entorhinal cortex and dentate gyrus, respectively. The granule cells of the dentate gyrus receive their input from the perforant path as well. This means that the input from the dentate gyrus to the CA3 area is a modified version of the information this area also receives directly from the entorhinal cortex [19].

Within the hippocampus the received information is further processed until the different kinds of sensory stimuli are combined into a representation of the current situation and environment.

The output from the hippocampus is widely distributed in the brain, either via the fornix-fimbria system or directly to numerous parts of the brain, thalamus, hypothalamus, motor and sensory areas in the brain stem and entorhinal cortex among others [14]. In terms of long term memory returning the processed information to the cortical areas it originated from can be a part of memory consolidation in the neocortex [18].

Whitlock et al. suggest that the output from the hippocampal formation is also sent to, and utilised by, the posterior parietal cortex, where it is believed to be translated together with information from other cortical areas into coordinates in reference to the body [20].

## *The functional cells of the hippocampal region*

Many cells in the hippocampal region are spatially modulated. This means that their activity is depending on spatial parameters like the animal’s position in the environment or the way it is headed.

There have been done extensive studies on cells in the hippocampal formation and entorhinal cortex, and several different types of spatially modulated cells have been identified and their properties examined.

There are four main types of functional cells in the hippocampus; the place cells, theta cells, head direction cells and grid cells.
The place cells were discovered first, by O'Keefe and Dostrovsky in 1971 [6] and were defined as complex spike cells by Ranck in 1973 [21]. In his study he also defined the theta cells. He went on to discover the head direction cells of the postsubiculum in 1984 [22].
The newest discovery was that of the grid cells of the medial entorhinal cortex, they were described by Fyhn et al. in 2004 [23].

## Theta cells

Theta cells are interneurons whose firing is in phase with the theta rhythm. This rhythm has been shown to be generated in the medial septum and it spreads to many structures, among other the hippocampus [15]. The theta rhythm is a spontaneous rhythm that can be recorded in the hippocampus in particular behavioural states, mainly arousal, attention or movement [24]. Even if it is most prominent in the hippocampus it can also be recorded in other parts of the brain [25]. Theta cells' action potentials are narrower than the ones of the complex spike cells and they only fire single action potentials [15].
Theta cells are generally assumed not to have spatial properties; however some modulation has been reported. Still their modulation in firing rate by location is much smaller than their modulation by movement [26].
The theta rhythm is postulated to have one or two out of three possible functions: 1) Binding together the activity of the entire hippocampal formation and possibly coordinating it with the activity in other parts of the nervous system. 2) Providing control over plasticity changes, making the development of LTP more likely when a cell fires in a specific phase of theta, and LTD more likely in a different phase. 3) Representing an ideal clocking system for single spikes in the pyramidal cells of the hippocampus because of its rhythmicity [15].
The complex spike cells in the CA1 or CA3- area have been shown to fire in accordance with the theta rhythm (see: *Phase precession*).

## Place cells

Place cells are spatially modulated cells. They have been found in both the CA1 and the CA3 area of the hippocampus as well as in the dentate gyrus. The place cells in the dentate gyrus are granule cells while the place cells in the hippocampus are pyramidal cells with the ability to fire so called complex spikes. Complex spikes are defined as multiple spikes with short inter spike intervals [21]. The later spikes typically have smaller amplitude and a longer duration than the first. The inter spike interval will also become gradually longer as more spikes are fired. Place cells have low background firing rate, firing selectively in their place fields [2, 7].

The most striking feature of the place cells is their ability to be active in only a specific part of an environment, coding for the animal's position in space.

The properties of place cells will be described later.

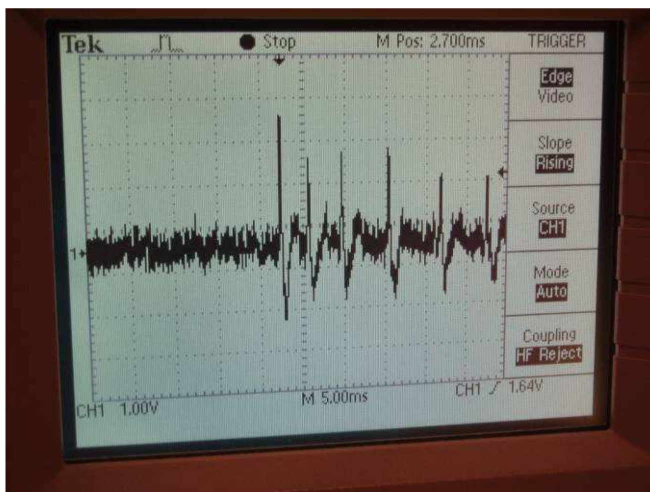



**Figure 6. The firing pattern of a complex spike cell registered using tetrodes lowered into the CA1 area of the hippocampus.** This registration was done during an acute experiment where the rat was anesthetised using urethane. The picture is taken of the oscilloscope connected to the recording equipment. Note the decrease in spike amplitude and the increase in inter spike interval.

## Grid cells

Grid cells are located in layer II and III of the medial entorhinal cortex (MEC), and like place cells they are spatially modulated, however unlike place cells they do not signal for one specific location. A grid cell's firing fields make a regular, triangular pattern that encompasses the entire environment explored by the animal. These grids are most likely the metric basis of the place cells' firing fields [23].

The firing fields of the most dorsolateral grid cells have the smallest and best defined firing fields. It is the cells in the dorsal parts of the MEC that project to the place cells in the dorsal hippocampus, which are the place cells with the best defined firing fields [27].
Grid cells realign when they are introduced to a new environment. When grid cells realign, place cells undergo global remapping. In contrast to place cells grid cells co-localised in an environment shift their fields in concert; it seems that cells that are coactive in one environment will be active in another environment as well [28]. When changes are made to an environment the orientation of the grids change in accordance to the changes in the environment, but their intrinsic spatial structure remains unchanged. Based on the fact that place cells remap when grid cells realign it is possible that these two occurrences are initiated by a single process triggered by the same sensory information [28]. This information is probably passed on from the entorhinal cortex to the place cells of the hippocampus [18]. Still, during rate remapping or partial remapping of place cells (see: *Place cell remapping*) the grid cell activity stays the same. This indicates that the hippocampus needs input from other sources in addition to the MEC.

### Head direction cells

Head direction cells are cells that fire selectively when the rat's head points in a specific direction within an environment.
They are mainly located in the presubiculum and anterior thalamus [29]. Head directions cells use landmarks to maintain their orientation, however they may also rely on path integration if the cues are removed [30]. Head direction cells may also respond do non-spatial parameters. For instance Taube et al found that some cells could fire robustly in one pass of the head in the preferred direction, but on the next pass they fired sparsely. This implies that head direction is not the only input to these cells [31]. The MEC contains cells that show both grid cell properties and are modulated by head direction, these have been named conjunctive cells [32].

### Connectivity of the major functional cell types of the hippocampal region

The functional connectivity between the different types of cells in the hippocampal formation seems simplistically to be that the head direction cells provide input to the grid cells in entorhinal cortex which in turn give input to the place cells in the hippocampus [15].

How the symmetrical pattern of the grid cells give rise to the focal, non repetitive patterns of the place cells remains unclear. Likewise how much influence the place cells have on the head direction cells or the grid cells.

## *The properties of place cells*

### Place fields

The part of an environment in which a complex spike cell fires significantly more than in the rest of the environment is defined as the cell's firing- or place field. It has been shown that the cells located in the dorsal hippocampus have the smallest and best defined place fields as compared to the fields of the cells located in the ventral part [27].
Within its firing field the place cell exhibits some distinct activity, in particular a significant higher firing rate inside the firing field than outside, and also a decrease in firing intensity with the increasing distance to the centre of the place field [7, 33]. Some cells may also have directional firing tendencies, the firing rate increasing when the animal makes a turn for instance to the right [33]. Breese et al. 1987 also found that the place cells showed a slight preference for firing on the way into a place field rather than moving out of the field [8], on average the number of spikes within a burst increases towards the middle of the field, and decreases on the way out [34]. In a polarised environment, like a track or a maze, place cells may also develop firing fields that are only present when the rat is traversing the field in a specific direction [35] or have a decreased firing rate if the rat traverses the field in a nonoptimal direction [8].
Place cells are not restricted to having place fields in only one environment, most place cells seem to have place fields in multiple environments [7, 35]. Typically about 50% of place cells active in one environment will also be active in a second environment [36].
Two place cells located close to each other in the hippocampus may have place fields close to each other in one environment and far apart in another. Based on this observation place cells are not thought to make a topographically organised map of the environment [35, 37].
In a relatively stable environment place fields remain stable over long periods of time, Thompson et al. recorded stable place fields over extended periods of time, up to 153 days [38].

## Non spatial properties of place cells

Place cells are known to be spatially selective, but they do not only encode the animal's position in space. Their activity may also help encode other parameters, like speed, direction, turning, and they can also reflect on non spatial parameters, like the specific behaviours of the animal [8]. The categories of stimuli that affect the hippocampal principal cells can be divided into spatial and non-spatial. Non-spatial stimuli can for instance be odours, objects, visual cues, sounds, or, at least for primates and humans, faces and pictures of scenes. Spatial stimuli entails information like speed, directionality and position [15].

Goal cells have also been described, they can be active as the rat runs from start to the goal arm [35], and stop firing after the reward placed in the goal arm is retrieved [39]. These goal cells may also shift their firing fields if the goal location is changed [33].

The idea that there are two distinct classes of place cells, one responding to spatial orientation and one to non-spatial stimuli, has been shown to be incorrect. A place cell may very well respond to spatial stimuli in one environment and non spatial stimuli in another [8, 40].

## Motivation and previous experience

In addition to code for the environment surrounding the rat, place cells may also represent the rat's perception of its surroundings.

The firing properties of place cells implies that the animal is able to identify separate places in the environment, probably based on multiple sensory stimuli [41]. When other kinds of stimuli than spatial stimuli can modulate a cell's activity, the cells' representation of the environment will not only reflect on positions in space, but also the significance of the location. This was shown by Hölscher et al, among others. They found that a significant amount of place cells recorded in an eight armed maze showed increased firing when the rat was entering a baited arm compared to when the arm was revisited after the bait had been eaten [39]. This tells us something about the rat's experience in the maze; the rat assumes no new food is being placed in the arm once the previous one has been eaten.

If the rat's access to food is based upon a navigational task, like finding a specific unmarked localisation on an arena, then the likelihood of stable place fields emerging that are dependent on both the extra maze and intra maze cues increases [42]. This was shown by Zinyuk et al. when they taught one group of rats to find an unmarked spot on a rotating arena and another group to forage for food randomly scattered on the arena when it was stationary. The rats

taught the spatial task were more likely to have cells showing spatially modulated firing in reference to the intra-maze frame, the extra-maze frame or both. The control group had cells with clear firing fields when the arena was stationary, but they did not have any clearly defined place fields when the arena was rotating. These findings indicate that they had developed no place fields in reference to the intra-maze frame. These findings show that previous experience in an environment as well as the kind of information needed to solve a task influence the place field development [42].

### Distal and proximal cues

At first spatial localization was thought to be based solely on distal cues, any proximal cues having no significance, as shown by Morris in 1981 [43]. Results from later studies strengthened his theory. They showed that changing proximal cues, but not distal, caused no change in place field position and stability. When distal cues were changed, but not proximal ones, place fields would move or disappear [7, 35].
When rotating the distal cues, for instance a cue card, the place fields will rotate accordingly [33, 35]. However distal cues might loose their control over place field positioning if the subject learns that the cue is not reliable e.g. if the rat sees the cue card being moved [44]. Rotating proximal cues (objects in the test arena) does not cause any shift in place field positioning, the only exceptions are place fields located close to the proximal cues. They may partially follow a shuffling of objects and not respond to relocation of distal cues [45].
The findings of place fields located close to or surrounding objects which responded to manipulations of these proximal cues has led some researchers to define a new group of spatially modulated neurons. In one for their studies Rivard et al. placed a barrier in the enclosure. When rotating the barrier or removing it they noticed that the cells with firing fields close to the barrier was affected while cells with firing fields away from the barrier were not [46]. These studies, and others, imply that the distance between the firing field and the cue controlling it affects the amount of influence the cue has on the place field's properties. It has been shown to be the case for both objects [45, 46] and visual cues [47]. Rivard et al. [46] defined the cells that responded to the relocation of an object by shifting their firing field accordingly as object cells. Object cells' firing fields are attached to an object, not distal cues, and follow the position of that object irrespective of the object's position in the environment. Like goal cells or mismatch-cells they are place cells that represent parts of the environment that are not necessarily defined by their position in space,

but by their significance in a non-spatial context. The existence of objects cells makes sense; an efficient way of navigating is by using the known distance relationship that exists between two objects. By having a subpopulation of cells dedicated to map out the positions of salient objects their location can be used efficiently while moving about in an environment.
When assuming that distal cues are responsible for the development of stable place fields one would expect it to be impossible for place fields to remain stable if the distal cues were removed. This was shown by O'Keefe and Conway [35]. In contrast Muller and Kubie [48] performed a study where removal of the cue card did not cause place field disruption, in stead the firing field rotated in a random fashion. The removal of the cue card did cause the field to become slightly less defined.

### Spatial frames

Within a complex environment place cells may fire in accordance to different reference frames. Gothard et al. showed this in a study where the rat was taught to walk out of a box and to a goal location [49]. When the box, the goal location or both were moved in reference to the surroundings or each other three separate subpopulations of cells were found. One goal or landmark related population, one box- related population and finally location related cells (classic place cells). Subpopulations represent the environment in reference to different frames. The finding of these subpopulations might suggest that the hippocampus gives several representations of a single environment. The complete map of an environment is probably the sum of the representations given by the subpopulations.

### Path integration

It is assumed that both external and internal cues contribute to navigation. There are two main strategies for navigation; the use of landmarks and the internal registration of direction, speed etc. Path integration is the process in which this internally registered information derived through for instance the proprioceptive and the vestibular systems is used to calculate the position of the animal [50]. This kind of information is known as idiothetic information.
Place field location can be based on external cues, path integration or a combination of both. For shorter periods of time path integration can maintain stable place fields, nevertheless, for longer time periods without access to other cues, such as olfactory cues, the place fields will become progressively more unstable and start drifting [41]. This is partly due to the

accumulation of errors in the calculations made in the path integration. This effect is especially apparent in open environments, whereas a more complex environment, like a radial maze may give enough information to maintain the fields longer [51].
In real life the exteroceptive cues, like landmarks, will be used to navigate. Path integration will be available, but not consciously. Using idiothetic cues the brain can calculate, to a certain degree, how far the animal has moved, in which direction and at what speed. An important difference between idiothetic and exteroceptive cues is that exteroceptive cues can be used to generate a path from the current location to an unvisited location while idiothetic cues can only be used in previously visited areas. The path integration alternative is always available while the exteroceptive “mode” requires looking for landmarks and calculating the best path. In moments of danger the path integration alternative will be the most efficient one.

### Place cell remapping

Place cells have plastic properties and are therefore able to develop new place fields when introduced to a new environment or when a change in an environment occurs. When this phenomenon takes place it indicates that the hippocampus, and therefore most likely the rat, considers its surroundings to be different from previously.
Remapping can be expressed in two ways, rate remapping and field remapping. Field remapping can be divided into partial and global remapping.
Remapping may occur when entering a novel environment or as a result of changing or rearranging the spatial cues. It may also take place in response to non spatial changes, like changes in context.
Muller et al. [48] were the first to define remapping, by manipulating single aspects of the environment and registering the change in place field activity. They found that changing the shape of the testing enclosure caused active cells to either stop firing or establish a place field at a different location. This was defined as a global remapping. When they increased the size of the enclosure some cells remapped, and those which did not increased the size of their firing fields stayed in the same position. This was defined as a partial remapping [48].
Partial remapping may also occur as a result of non-spatial changes, for instance when the goal location in a familiar environment is moved. If there are water wells available on several locations in an environment, changing the setup to one where water is only available in one well can make a place cell’s firing field move to the well containing water or to cover the path

towards the it [33]. Here the remapping is not due to changes in the spatial cues, but to changes in context.
Non spatial changes may also cause a rate remapping. For instance when changing the colour or shape of the testing enclosure [52]. Such a change in context is usually followed by a rate remapping, where there is no change in location of firing fields, but a significant increase or decrease in the firing rate [27]. The degree of remapping depends on how big the change is. Some changes might elicit both rate and field remapping.
When remapping globally the CA3 neurons seem to have a more significant change in both field and rate remapping than CA1 [52].
One theory on the importance of both types of remapping is that partial or rate remapping allows for representation of different experiences in the same spatial frame whereas global remapping makes it possible to distinguish two similar episodes in different spatial contexts [52].
The fact that remapping occurs as a result of changes both in reference to the spatial frame or to the context makes remapping important for both spatial and episodic memory. It allows us to remember both the place of importance and why it was important. It also reduces interference between similar memories [53].

## Functions of and mechanisms for remapping

The process of remapping is a plastic process. Place cells can be active in multiple environments and “silent” in others [7, 41, 54]. This combined with the fact that no subgroups of place cells that are always active together have been found, allows the hippocampus to represent a very large number of environments and give each environment a unique representation. Partial remapping and rate remapping give the hippocampus the ability to discriminate between two slightly different versions of the same environment.
Global remapping in the CA3 comes about together with grid realignment in the MEC. The grid cells in the entorhinal cortex will shift their firing fields according to spatial changes that also cause place cells in the CA3 to remap globally. However when the cells in CA3 undergo a rate remapping the grids of the cells in the entorhinal cortex remains unchanged [28]. The same can be seen when the cell of the CA-fields undergo a partial remapping [36].
Different theories try to explain how the structured firing pattern of the grid cells can give rise to the seemingly random firing patterns in the hippocampus. Several theories postulate that a

place cell will fire when its grid cell inputs overlap. The transition from the symmetrical pattern of the grids to the seemingly random distribution of place fields is explained by the fact that the scale of the grids increase from the dorsal to the ventral part of MEC. If a place cell receives input from grid cells with different spatial scales this would cause the cell's place fields to appear in an unsymmetrical pattern [55]. However this theory does not explain partial remapping since there is no evidence that grid cells can partially remap, while place cells do. This has been taken into account in the contextual gating theory presented by Hayman and Jeffery [36]. Their theory is based on the processing of information in the dentate gyrus. As previously mentioned CA3 received the input from MEC directly as well as through the granule cells of the dentate gyrus [19]. The gating theory proposes that the signal from MEC to CA3 that goes through the dentate gyrus is gated based on contextual conditions. This will cause parts of the signal to reach the cells in CA3 while other parts will disappear. Changes in context will cause other parts of the signal to reach the CA3 than previously [36]. Rate remapping is apparent in the dentate gyrus and the CA areas [19]. This suggests that the rate remapping in the dentate gyrus might cause the active place cells of the hippocampus to change firing rate.

Wills et al. used a so called morphing experiment to examine the mechanisms of remapping. First they introduced the animals to a square and then a circular testing apparatus to establish the place fields. Next they recoded cell activity in a testing apparatus that could be morphed from a square to a circle. The rats were tested in the intermediate shapes in a random order. They showed that there was no gradual remapping between the circular and the square shapes. There was a point in which all the place cells they registered in CA1 remapped simultaneously, indicating that pattern separation and completion mechanisms might be involved [56] (see: *Pattern completion/pattern separation*).

A similar study was conducted by Leutgeb et al. which also morphed between a square and a circular enclosure; however they registered place cells in both CA1 and CA3. The rats were tested in the intermediate shapes from circular to square and from square to circular, making the change gradual. They found that a rate remapping occurred in the intermediate shapes [57].

These studies show that the plasticity of the place cells does not cause permanent changes in their properties. A remapping, of firing rate or field, might be readily undone by reintroducing the rat to the previous environment [56, 57].

### Phase precession

Phase precession is a progressive advance of the firing time of a place cell or grid cell in relation to the theta rhythm when the animal traverses the cell's firing field [58].
Phase precession was first recorded by O'Keefe and Recce in CA1 place cells in 1993 [34]. When traversing its place field the complex spike cell fires at a frequency related to that of the theta rhythm in the hippocampus. When the rat enters the cell's firing field the place cell starts firing in bursts and it continues to fire as long as the animal remains in the cell's place field. The cell fires the bursts with a slightly higher frequency than the theta rhythm, which results in the spikes being fired on a slightly earlier point in the theta cycle for each burst as the rat traverses the firing field [15, 34].
A cell will always fire its first burst at approximately the same phase of the theta cycle, but the starting point varies from cell to cell [34]. Phase precession makes it possible to determine more accurately where the animal is located. When considering the place cell firing it is possible to conclude that the animal is standing somewhere in the cell's firing field, but when considering the cell's firing in relation to the theta rhythm it is possible to determine in which part of the place field the animal is located.

### *Pattern completion/pattern separation*

The notion that the hippocampus functions as an index for memories stored in other parts of the brain leads to the theory of pattern completion and pattern separation. When a room closely resembles a previously visited room there needs to be a system for recognizing if the room is in fact the same room that was visited earlier or a different room. This is believed to be possible because the massive amount of information from the neocortex is converged on a smaller number of neurons in the hippocampus. Pattern completion is dependent on the information from the neocortex to activate the same neurons in the hippocampus as the previously recorded memory did. Pattern separation is the opposite process, the input from the neocortex activates a different group of neurons, and a new activation pattern is established [59].
Evidence for pattern separation is that place cells are able to undergo global remapping when only parts of the sensory input are changed, whereas evidence for pattern completion is that place fields can maintain their firing fields after removal of some of the landmarks that defined the environment originally [58].

One theory on the mechanisms for pattern separation is that the modification of information from the MEC to the CA3 in the dentate gyrus combined with the strong synapses and sparse firing in the granule cells makes a strong enough signal to CA3 that might help separate similar patterns of activity received by the CA3 from the entorhinal cortex [60]. This is consistent with the gating hypothesis of Hayman and Jeffrey [36] described earlier.
Pattern separation occurs throughout the brain, among others in the sensory systems, it is not a mechanism specific to the hippocampus [53].

## *Aim of the thesis*

Memory is an abstract concept, it can not be directly observed. However, one can observe the effects of certain types of memory as a change in behaviour. The behavioural change can be monitored by giving the test subject a task to perform, like Breese et al.[33] , teaching a rat that approaching a specific water cup in the test area would fill the water cup with water, giving the rat access to water. It can be also be used to look at the formation of memory at a neuronal level. The development of a firing field or an alteration of the firing pattern of a place cell is a change in the cell's "behaviour". This is of course a simplification of the very complex neuronal network of the hippocampus, other cells, in addition to place cells, are needed to maintain a spatial memory. Still it shows us that the neuronal network has somehow formed a memory of that specific place, making the cell fire every time the test subject enters the place field.
By recording single cell activity extracellularly one can register the cell activity over a longer period of time, also in awake and freely moving animals. This makes it possible to register both the cell activity and linking it to its behavioural correlates.
Motivation is an important factor in learning, and as described previously motivation is one of the factors that might influence place field generation and stability.
Several studies have shown that place cell activity can be modulated by reward; cells may for instance fire selectively as the rat runs into the goal arm of a maze [35, 39] or a firing field might move in reference to the location of a reward [33].
Most studies done concerning place cells, studying freely moving rats, have used a reward as a motivational factor. This has been done mainly to increase the rat's activity level in the environment and make sure it traverses the entire testing apparatus, which is important for optimal recordings. This approach has helped provide much of the current insight into the

properties of place cells, among others what they might encode for, what makes them remap and their stability. What have not been studied at any great length is if place fields can be stable in the absence of reward as a motivational factor. Furthermore, if the place field would respond to changes in the environment in an expected manner compared to previous studies. The aim of this study was to see if stable place fields would develop in freely exploring rats when no reward was given to encourage this activity. Furthermore, would the place fields that developed show the expected place field properties? To increase the rats' activity level in the no-reward condition objects were placed in the apparatus.

# Materials and method

Parts of the procedures described here can be quite time consuming and difficult to do with only one pair of hands. Therefore the practical project was a corroboration between me and another master student, Brita S. Fiskå. Therefore the results presented in our master theses are identical. The writing of the master thesis was done individually.

The procedures for the making of tetrodes, loading the microdrive, implantation of the microdrive and lowering of tetrodes is briefly described in this section. The complete procedures can be found in the lab protocol written by Jelena Mrdalj as a part of her master thesis in 2006.

## *Pilot project*

It is known that place cell activity can become goal oriented, and therefore the cells may for instance become active when a rat walks into the arm of a maze which contains a reward [35]. We wanted to see if this goal orientation would appear if the goal was something abstract and movable, like a beam of light. We therefore tried to link the beam of light to a reward. Would a place field develop that became active when the rat reached the lighted area, and would the place field remap when the lighted area was moved?

Rats without implanted tetrodes were placed in the enclosure and a piece of a chocolate cereal ring was dropped on the floor in the enclosure. A small flashlight was then used to light up a limited area surrounding the reward. We started with the area being lighted till the rat found the reward, then we limited the period the area was lighted for, the aim being to teach the rat that a flash of light lasting a second of two meant a reward was placed in the spot where the light had been. We soon discovered that due to the rats' good hearing and night vision they could hear, or see, where the reward was being put on the floor. This made it virtually impossible to define if any remapping was due to the light beam or the sound, or sight, of the reward being put on the floor. The project was abandoned.

## Main project

### Subjects and housing

For implantation of electrodes and recording of place cells: Male Long Evans rats from Harlan Animal Research Laboratory, UK.

All the rats used in this study arrived at the Institute of Biomedicine animal housing facility the 9th of July 2008.
Their weight at the time of surgery varied between approximately 400 and 600 g.
Before implantation, while they were being handling and trained the rats were housed in the Department of Biomedicine animal facility where they were kept in groups of four rats. The cages were kept in standardised conditions, temperature 21°C at a 12 hour light/dark cycle. The rats were given free access to food and water till they reached the minimum required weight for implantation. After the minimum weight was reached their food access was restricted.
After implantation and during the testing period they were kept in cages measuring 60x40x30 cm. Two rats were kept in each cage separated by a transparent plastic wall on which their water bottles were located. This wall allowed the rats to see and smell each other but prevented them from gaining access to the other rat which might cause their implants to get damaged.
The cages were kept in a Scantainer (*Scanbur AS, Denmark*) with a humidity of 60-65% and a temperature of 22 °C.
Their bedding was changed once a week and they received food on a daily basis. One a week they were weighed to make sure their weight was stable.

### Handling and training

When the rats arrived they were not used to being handled, and for approximately three weeks after arrival their cages were moved once a day to a room in proximity to the housing room. Here each rat was handled for a couple of minutes and then put in a circular enclosure containing bedding and a range of different objects. They were also given reward in the form of chocolate flavoured cereal. The goal was to get them comfortable with being handled and to be relaxed while sitting still on a person’s arm.

## Making tetrodes

Tetrodes were made from platinum wire containing 10 % iridium (*California Fine Wire, USA*). The wire used was either 17 or 25 µm in diameter.

A roughly 25 cm long piece of platinum wire is folded into a long loop with the ends stuck together with adhesive tape. The loop is then fastened over a magnetic stirrer in such a way that two loops are formed. In the lower end of the two loops a magnet was fastened. By using

the magnetic stirrer the loops were twined together making a tetrode consisting of four electrodes. The tetrode was then heated, with a heat gun at 230° C to make the electrodes stick together. Afterwards the tetrode was cut loose and was ready to be loaded into a microdrive.

For further details see lab protocol.

## Loading the microdrive

Each microdrive was loaded with 4 tetrodes, 16 electrodes. The untwined end of the tetrode was burned to remove the insulation, and each electrode was fastened to the microdrive by carefully winding them around contact pins on the microdrive.

Silver paint was used to secure good contact between the electrodes and the contact pins on the microdrive. The area of the microdrive where the connections between the electrodes and the contact pins were made was covered with nail polish to prevent it from getting damaged.

The resistance in the electrodes was measured, and if it was too high the electrode received a layer of platinum by lowering the tetrodes into a platinum solution and administering a brief current. The aim was to obtain a resistance between 200 and 500 kΩ. The strategy applied was that any electrode with a resistance < 600 kΩ was platinised, aiming to get the resistance to approximately 400 kΩ. This was due to the fact that we only platinised once and the effect of the platinising varied.

The microdrive was also checked for any short circuits between the electrodes using a Flute multimeter (*John Fluke mfg. co, inc, USA*).

Most of the microdrives used for implantation were loaded with 25 µm diameter electrodes.

For further details see lab protocol.

## Implantation of the microdrive

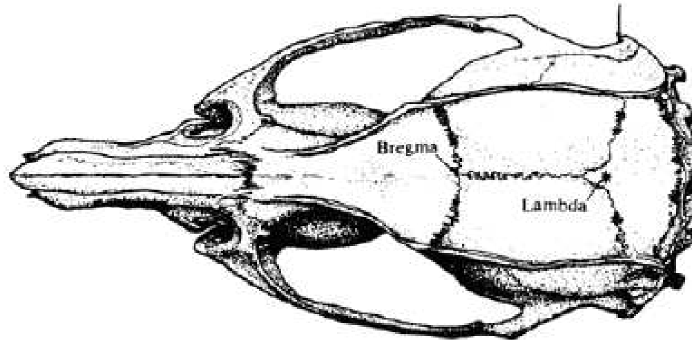



**Figure 7. A dorsal view of the skull of a male Wistar rat.** Bregma and Lambda are indicated by arrows. Adapted from Paxinos et al. [61]

At least one day prior to surgery the rat was transported from the animal housing facility to the laboratory, located in gray zone. Here they were housed in the Scantainer for at least one night to allow them to acclimatise and were also kept there during the testing period after implantation.
For implantation surgery the rat was first anesthetised in an Induction Chamber (*SurgiVet/USA*) using Isofluoran (*Isoba vet., Schering-Plough Animal Health, England*) mixed with oxygen and NO. When the rat had fallen asleep it was removed from the chamber and Equithesin (*Apotek 1 Svanen, Bergen*) (0,4 ml/100g) was injected intraperitoneally. The first injection was usually approximately 80% of the maximal dose. Before surgery began the back foot reflex was checked to make sure the rat was deeply anesthetised. If the reflex was still present the rat was given an additional dose of Equthesin, between 0,2 and 0,5 ml depending on how strong the reflex was and the rat's weight.
The top of the head was shaved to prevent fur from entering the wound causing an infection, and the exposed skin was disinfected using Iodine solution (*Haukeland Sykehusapotek, Bergen*). The eyes of the rat were covered with Viscotears eyegel (*Novartis Healthcare AS, Denmark*) to prevent them from drying out or any pieces of bone or cement to damage them. The rat was placed in the stereotactic apparatus intraaurally. An incision was made medially on the skin of the skull and the bone sutures of the skull were exposed. A hole for implantation of the tetrodes was drilled in the skull in relation to bregma, 3,8 mm posterior and +2/-2 mm lateral. The exposed part of the outermost of the meninges, the dura was

removed carefully, using a bent syringe needle, making sure to cause minimal damage to the underlying cortex or cause a bleeding.
Three screws were fastened into the skull, two in the frontal bone, and one in the occipital bone. One of the anterior screws was used as a reference and connected with a short wire to the microdrive, the others screws helped fasten the microdrive to the skull. The screws were covered with a thin layer of dental cement (*Grip Cement, Dentsply Inc, USA*).
The tetrodes were lowered into the brain, usually 1500- 1800 μm, and the hole in the skull covered with Spongostan Coagulating gel foam (*Ferrosan, Denmark*). The outer cannula was lowered onto the gelfoam, and the lower ¾ of the cannulas was coated with heated, sterile Vaseline (*Nycomed Pharma, Oslo, Norge*) to prevent the cement from sticking to the cannulas which could restrict the lowering of the tetrodes. Finally the microdrive was securely cemented onto the skull using acrylic dental material (*Swebond, USA*). The rat was removed from the stereotactic apparatus and the front or back of the incision was stitched up if needed. Because rats tend to have laboured breathing after Equithesin anaesthesia the rat was put in the Induction Chamber again immediately after surgery, in the flow of oxygen.
After the rats woke up from anaesthesia they were given water in a bowl and fed soft food.
A anti bacterial cream, Fucidin (*Leo Pharma AS, Oslo*) and local pain relief Xylocain cream (*AstraZeneca AS, Oslo*) was applied to the operation wound twice a day the first one to three days after surgery. After this Bacimycin powder (*Actavis, Iceland*) was sprinkled on once or twice a day until the wound had started healing, was dry and no signs of infection were seen. For standard implantation procedure see lab protocol.

## Lowering of the tetrodes into the brain

The tetrodes were lowered in small steps once or twice a day, starting three to four days after the implantation. Sessions were initially performed in the circular apparatus to search for complex spike activity. These sessions were also important because they allowed the rat to get used to being connected to the headstage. Depending on the initial depth of the tetrodes, and the signal seen in the session performed that day, the tetrodes were lowered between $^{1}/_{16}$ or ¼ of a rotation, i.e. between 12, 5 and 50 µm. This was done by turning a little screw on the microdrive counter-clock wise (see lab protocol).
To reach CA1 the tetrodes needed to be lowered about 2,2 mm down from the surface of the cortex according to the Rat brain atlas [61]. By doing the lowering of the tetrodes gradually you minimise the damage done to the brain tissue.

# Testing room and equipment

## Testing and recording room

The same room was used for the testing during lowering of the tetrodes and recording sessions.

The room measured approximately 4x 4 m.

The apparatus was placed in the middle of the room and was elevated 70 cm above the floor. It was surrounded on all four sides by textile curtains. The curtains were placed approximately 1 m away from the testing apparatus. The curtain enclosure measured approximately 3 x3 m. The lights were located outside the curtains, and the room was kept semi dark during recording sessions.

A black plastic disk was suspended in the ceiling above the apparatus. A camera was placed on a modified camera holder inside the disk, a monochrome camera (*Dinion CCD camera, Bosch, Germany*) with a wide angle lens (*Computar, Japan*). The camera was connected to the tracking unit, providing the tracking of the rat's position. The camera was focused and adjusted to show the entire apparatus below.

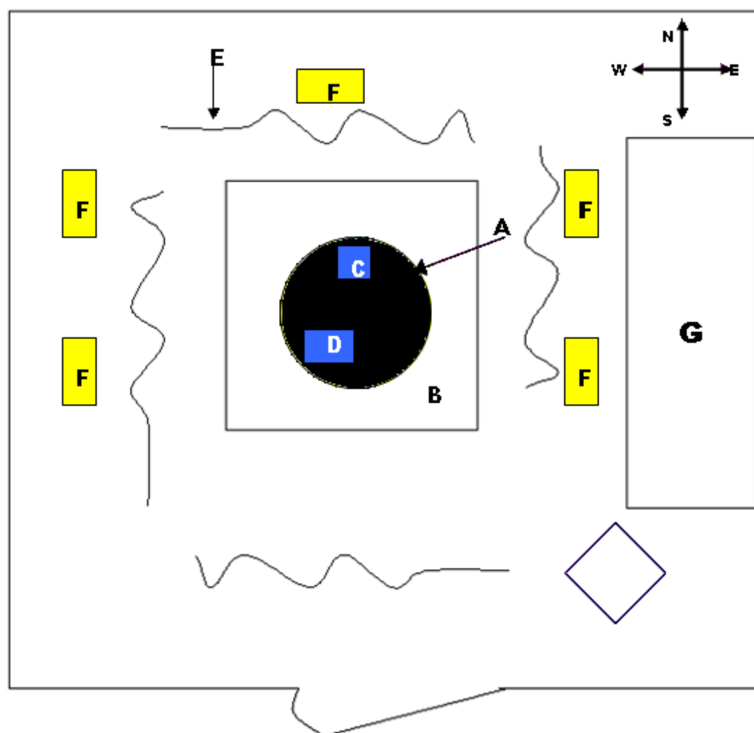



**Figure 8. A schematic drawing of the testing and recording room.** The A on the figure indicates the black Plexiglas disk; B the testing apparatus; C video camera; D recording system preamplifier; E curtains; F lights; G recording equipment, computer, monitors etc.

## Recording equipment

All data recordings, as well as position tracking was done using Axona Dacq Recoding System.
The technical details in this section and a more detailed description of the system can be found in the Axona Dacq Recording System User Manual, Axona Ltd., UK. For a schematic overview of the system, see Figure 9.
A headstage (*HS-116*) is fastened to the microdrive implanted on the rat. The headstage is connected to the connector pins on the microdrive. Here the signal is processed by FET transistors with a gain of 1; the signal is not amplified, but the noise level is reduced. Via a light, elastic cable the signal is then transferred to the preamplifier (*PR-116*) located on the disk suspended over the testing apparatus. This cable was suspended a counter weight system made in the lab. This system ensured that the rat could move freely in the testing apparatus without the cable becoming too short, restricting the rat's movements or too long, allowing the rat to reach the cable.
In the preamplifier the signal is amplified x1000 and then each signal is divided into a differential pair, to minimise the risk of noise interference. Finally the signal reaches the main recording system, consisting of the system unit box and a Pentium PC.
The system unit box can process signals from up to 32 channels, it contains amplifier/ filter units (*SC-104*), and each of these units processes the signal from four channels. The type of filter and the gain is set via the software on the computer. The signal is then digitalised, displayed on the computer screen and stored as a computer file if wanted. The signal from single channels may also be transferred to a speaker, making it possible to assess the spike activity converted to sounds, or the signal may be fed to a digital oscilloscope (*Tektronix TDS 224, Textronix, USA*). On the oscilloscope the trigger level can be adjusted, making it possible to inspect the recorded spikes to see if they are in fact action potentials, and if they originate from complex spike cells.

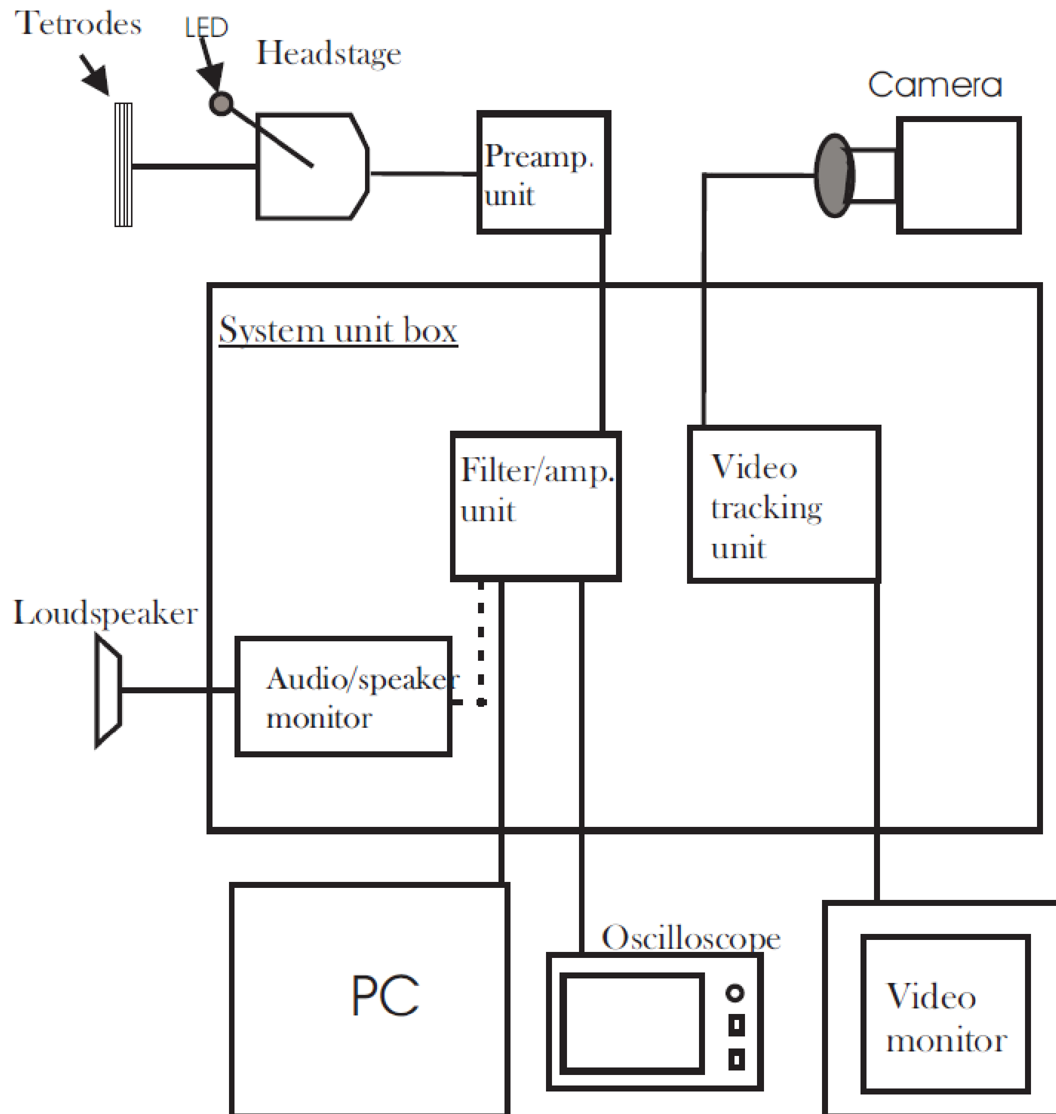



**Figure 9. A schematic drawing of the data recording system.** The signal registered on the tetrodes, and the picture captured by the camera is processed and distributed by the system unit box through various output channels in the form of computer files, picture and sound.

## Position tracking

The technical details in this section and a more detailed description of the system can be found in the Axona Dacq Recording System User Manual, Axona Ltd, UK.
The position tracking was done using the Axona tracking system.
Position tracking system consists of several parts. The light that is to be tracked is located on the headstage, which is connected to the microdrive. There is also the video camera, a video monitor and the video tracking unit, which is located within the system unit box.

The light used in this study was a LED which emitted infrared light. The camera was as previously mentioned positioned in the plastic disk located in above the testing apparatus. The signal was led through a separate wire than the signal from the tetrodes to the video tracking system located in the system unit box. In the video tracking system the x, y coordinates of the tracked light is recorded and relayed to the recording system PC.
The tracking system operated in a monochrome mode, which means the tracker locates either a dark spot on a bright background or a bright spot on a dark background. In this study the tracker was set to look for a bright spot on a dark background, the bright spot being the infrared light emitted from the LED.
The input from the camera to the video tracker was also displayed on a video monitor (*Hitron Systems inc., Korea*). This enabled us to assess the quality of the tracking, which could vary depending on the enclosure and the light conditions in the testing room. Adjustments could then be made to optimise the tracking by dimming the lights in the room or adjusting the brightness or contrast settings of the camera via the computer software. Via the software the size of the area captured by the camera could also be adjusted.
Finally the position tracking was saved as a computer file. In addition to the digital file of the tracking, a VHS video recorder (*Grundig, Germany*) was connected to the monitor, and the some of the sessions were video taped.

## Testing apparatuses

While the tetrodes were being lowered a grey circular fibre glass apparatus was used (90 cm in diameter, 50 cm in height).
Next two square boxes made of plywood were used (100 cm x 100 cm, 50 cm in height). The sides of the boxes were painted with transparent lacquer. The bottoms of the boxes were painted with mat, black paint.
All the test apparatuses had a cardboard (50x70 cm) attached to the wall. This acted as a cue card polarizing the enclosure, ensuring that the rat had the ability to orientate itself, which is crucial for the formation of lasting place fields.

## Recording protocols

### Test procedure

During the lowering of the tetrodes towards the CA1-area the recordings were performed in the circular apparatus. Here small pieces of chocolate cereal were randomly scattered to encourage the rat to explore. The north wall of the apparatus was marked with a white cue card.
Recordings were made when cells appeared, each recorded session lasted 120 or 180 seconds. When multiple spike cells were discovered the tetrodes were not lowered further and a session was recorded the following day to confirm that the cell activity and place fields were stable. After two consecutive days with stable complex spike cell activity in the circular container, the rat was tested in a square enclosure.
The search for cells with place fields was mainly done in the square apparatuses. The first square apparatus contained a combination of four objects out of five: a glass sphere, a paper box, a small can of paint, a metal block or a spool with metal wire. The objects were equidistantly spaced. The north wall was marked with a black cue card.
No reward was given in this apparatus.
The other square apparatus contained no objects, but reward was given. Chocolate cereal was sprinkled randomly in the area during sessions. The north wall was marked using a grey cue card. The sessions recorded in both these apparatuses lasted 300 seconds.

***Overview of the recording procedure***

1. Lowering of tetrodes and localization of complex spike cells in the circular apparatus.
2. Recording sessions in the square apparatus containing objects, making several changes concerning objects and object placement.
3. Recording sessions in the empty square apparatus where reward was given.

**Figure 10. The three testing enclosures.** Top picture shows the circular apparatus. The lower left picture shows the enclosure containing four objects and the lower right picture shows the empty enclosure where the rat received reward.

For one rat sessions in the circular apparatus showed very high activity on multiple tetrodes, and the data analysis showed that the cells were not spatially modulated. Sessions were then recorded in the square apparatus while the tetrodes were lowered further.

At least two sessions were recorded when changes in the environment were made. An exception was made concerning sessions recorded in familiar environments before or after sessions in novel environments.

## Data recording

The first time the rat was connected to the recording system a setup file was made. Depending on the amplitude of the signal, the gain was set between 20 000-30 000. Each tetrode was designated a reference electrode. The reference electrode was preferably one with little noise and spikes to ensure the least possible amount of interference from spikes and noise recorded on the reference electrode.

Filter mode was set to high pass (500-10 000 Hz).

The recording mode was set to B-A, with B being the reference electrode, and A the spike recording electrode. The signal from A, where the presumed spike was seen was subtracted

from the signal in the reference electrode, B. This reduces the background noise and inverts the action potential, recorded extracellularly, to the positive signal shown on the screen. Usually reference electrode and gain were set for the tetrodes; however it was possible to alter these parameters for a single electrode.
Before recording the session the trigger level was set. The trigger level is an amplitude threshold, which allows all spikes with amplitude above the set value to be recorded. The value is set using the signal from the channels of interest. The optimal trigger level is a level that allows for the spikes to be registered, but eliminates most of the background noise.
The tracking window was set to approximately the boundaries of the test enclosure, and contrast and brightness was set to ensure the best possible LED tracking. These parameters were also saved in the setup file.
Reference electrode and gain were adjusted during the lowering of the tetrodes if needed, and the tracking window was changed between sessions in the circular and square enclosures.

## Saving data

Registered results were saved using a filename consisting of the name of the rat and the session number. In addition sessions in a square enclosure were marked with an f, the sessions in the circular enclosure with a t.
The data was stored on a network server, and data analysis was performed offline using a different computer from the one used to do the recordings.

## Data analysis

To perform the data analysis Tint Cluster Cutting and Analysis Software Version 4.0 (*Axona Ltd*, *UK*) was used.

## Isolation of single units

Using tetrodes allows for registering of several cells simultaneously. It also makes isolating spikes from single cells easier, because forcing the electrodes together in tetrodes makes sure they are located in close proximity to each other and might register the same signals, only at a slightly different angle and distance.

The single units are isolated by spike sorting. In the Tint cluster cutting window the spikes recorded from a tetrode can be viewed as points in a scatter plot, see Figure 11.
Each tetrode is analysed separately. Amplitude is the standard parameter in the scatter plots, and the spikes are separated based on this parameter. For each tetrode six scatter plots are made using the data from the four electrodes based on this parameter. In the two dimensional scatter plots spikes originating from a particular cell will gather to form a cluster. Other parameters can also be used; for instance height of peak, height at time T or time of peak. Amplitude is usually the most suitable parameter for identifying a cluster while the others can be used to deselect spikes not belonging to the cluster. Using these different parameters a cluster is located, enclosed with a boundary and allocated a cluster number and colour. The program also shows the waveforms of the spikes within a cluster found on an electrode of the selected tetrode, as shown on the right side of the Figure 11. This is essential to confirm that the spikes enclosed in the chosen cluster are not artefacts and that they do indeed originate from one cell. It is also possible to superimpose the waveforms of spikes from two clusters simultaneously. This allows one to compare and conclude if the two clusters are in fact spikes originating from the same cell. Artefacts can easily be recognised due to their shape and amplitude being registered as identical on all electrodes, which spikes originating from a neuron do not.

## Autocorrelation

After defining a cluster the spike waveforms are evaluated. If they look similar the assumption is made that the spikes originate from a single cell. This assumption can be confirmed by means of autocorrelation.
Autocorrelation shows the inter spike intervals. If the cluster contains spikes with an inter spike interval shorter than 2 ms, the cluster most likely contains spikes from more than one cell. This conclusion is based on the fact that a cell’s refractory period is approximately 2 ms, and within this period a new action potential cannot be generated within the same cell. A second spike occurring within this period must therefore have originated in another cell.

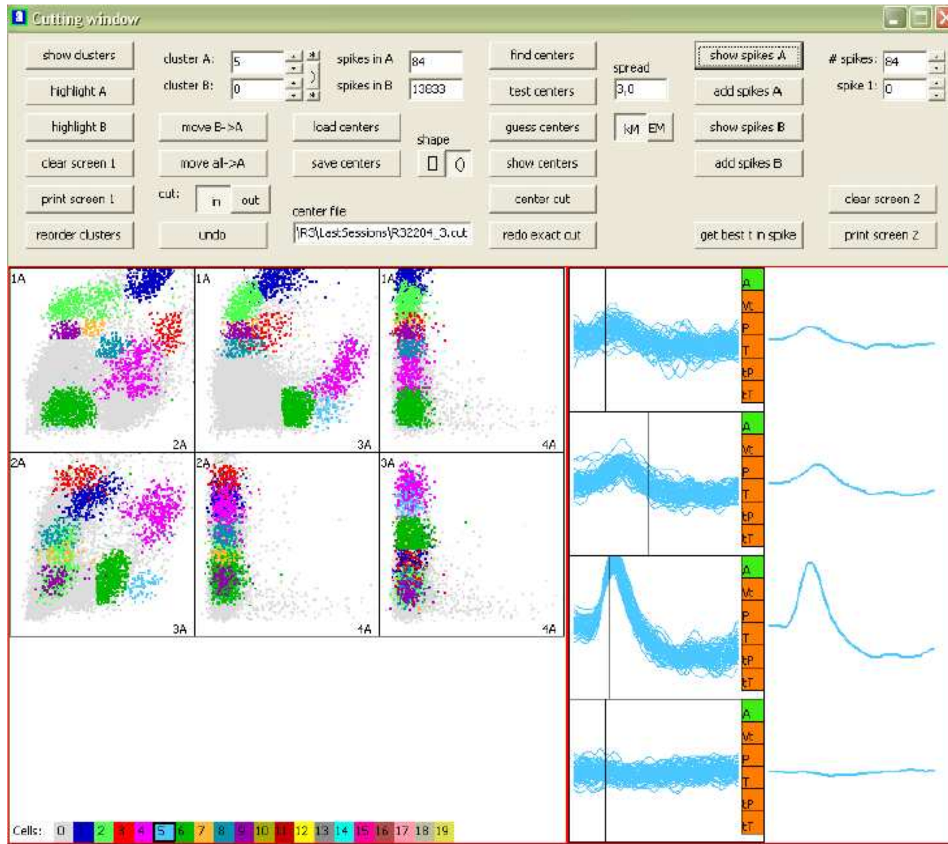



**Figure 11. Tint Cluster cutting window.** The left window shows the six electrode scatter plots with clusters colour marked. The lower panel shows the colour designated each cell. The right panel shows the spike waveforms of cluster 1. The left part of that panel shows the spike waveforms of 100 spikes in cluster 1, the right part shows the average waveforms of the spikes. The screen shot originates from the analysis of data from a session registered after the end of this study from rat R3. The data analysed originates from tetrode 3.

## Viewing spatial properties of cells

To examine the spatial properties of a cell the Tint field window was used. Here the spikes of a cell found in the Tint cluster cutting window could be viewed together with the tracking data registered in the same session. By superimposing the rat’s movements in the apparatus with the spike activity of a cell of interest it is possible to see if the cell becomes more active in a certain part of the environment, see left picture in Figure 12. To account for the variation in dwell time in the different parts of the environment a rate map is constructed, as seen in the right picture in Figure 12.

Rate maps are made by dividing the testing area into bins and summating the number of spikes occurring within each bin. The firing rate is calculated by dividing the number of spikes within the bin by the dwell time for this bin.This corrects for any uneven distribution of

spikes that is due to the rat not spending equal amounts of time in every part of the environment.

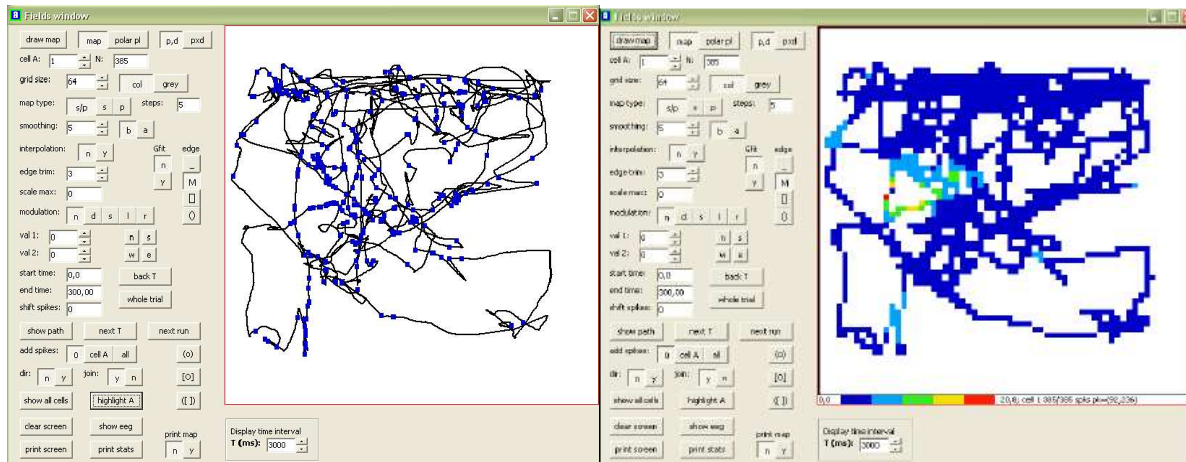



**Figure 12. Tint field window for cell 1 shown clustered in Figure 11.** The left picture shows the rat's trajectory, superimposed by the spikes originating from cell 1. The right picture shows cell 1's firing rate map. The bar below the firing rate map shows the scale used to show the firing rate, the red areas having the highest firing rate, the dark blue areas the lowest. The screen shot originates from the analysis of data from a session registered after the end of this study from rat R3. The cell was found on tetrode 3.

## Criteria for spikes recorded in different sessions being from the same cell

- The tetrodes must not have been moved between sessions
- The cluster and the shape of the spikes must look similar in the analysis program.
- The spikes must be recorded with the same electrode of a given tetrode.

A problem that might arise when using these criteria is that after longer periods of time the tetrodes might shift their position relative to the cells. This will cause a change in the shape of the spikes, and the cell might appear on another electrode than previously.

## Firing rate

To try to evaluate the overall activity level of a specific place cell the overall firing rate was calculated.

$$Overall\ \ firing\ rate = \frac{Number\ of\ \ spikes\ in\ cluster}{Duration\ of\ \ session}$$

## Perfusion and histology

When the experiments were completed a histological analysis was performed on the brains of most of the rats in order to localise the tetrodes' position in the hippocampus.

The rat was anesthetised using an overdose of Equithesin or Urethane (*Sigma-Aldrich Chemie GmbH, Germany*). The urethane was prepared for use in the lab. When the rat was deeply anesthetised it was perfused transcardially with saline mixed with heparin to remove the blood from the circulatory system followed by a perfusion with ca. 200 ml of either 4% formaldehyde or 4% paraformaldehyde. The brain was then removed from the skull and placed in the formaldehyde solution for at least 24 hours. It was then transferred to a 15% and 20% sucrose solution for saturation of the brain tissue.

The brain was then frozen using compressed $CO_2$ and cut in a Cryostat (*Leica CM 3050s, Leica Microsystems, Germany*) to 30 μm sections. The sections were transferred to adhesive microscope slides (*Superfrost Plus, Menzel GmbH& Co, Germany*).

The sections were stained using Cresyl Violet, a staining method which highlights the cell bodies. The staining procedure can be found in the lab protocol. The sections were then dehydrated and covered with cover glass (*Knittelgläser, Germany*) using Histokitt (*Assistent-Histokitt, Germany*).

Microphotographies were taken using a microscope equipped with a digital camera (*Nikon Eclipse 80i*).

After dissection of the brain from the skull the implant and the surface of the brain were inspected to determine if there were any signs of infection. The tetrodes protruding from the hole in the rat skull were examined to determine if they had been bent or damaged during implantation and lowering, and if they still formed a tight bundle.

We also tried, using a microscope, to measure approximately how far they were protruding from the skull and compared this to our calculations of the depth of the tetrodes in the brain.

# Results

In this section the results obtained in this study will be presented. First the testing procedures for the individual rats will be described and the effect changes in the environment had on the rats' behaviour. Next the place cells registered will be described, first their firing fields and how these reacted to changes in the environment, then autocorrelation and the overall firing rates of the recorded cells during the sessions. Finally the results from the histology will be reported.

## *Subjects*

A total of 15 rats were implanted with microdrives. The amount of data obtained from each rat varied; in some rats no cell activity was found, in others only a few cells were found and some had many. We found spatially modulated cells in very few rats, only 2.
All rats recovered from surgery. One rat lost its implant before neuronal activity had been recorded; this rat was put to sleep by giving it an overdose of Equithesin.
The results from sessions recorded with the 3 rats where we isolated cells that will be presented here.

**Table 1 The number of sessions recorded with each rat in the different testing apparatuses.**

| | Circular apparatus | Square apparatus | |
|---|---|---|---|
| Rat | received reward | containing 4 objects | no objects, received reward |
| B2 | 20 | 19 | 7 |
| R3 | 23 | 16 | 16 |
| G2 | 12 | 6 | - |

In general sessions were recorded in the circular apparatus when cell activity was found. If the cell activity seemed to be spatially modulated the lowering of the tetrodes were stopped and the experimental recordings in the square apparatuses started.

### Rat G2

Sessions were recorded in the circular apparatus, place specific activity was found and sessions in the square apparatus containing objects were initiated. In session 8 place fields were discovered in the apparatus containing objects. Two cells with place specific activity were found.
The place specific cell activity disappeared completely after session 11. We tried raising the tetrodes, but did not relocate the signal. Next we lowered the tetrodes further; the signal was however not relocated.

### Rat B2

Figure 13 shows the order in which the recordings from this rat were done.
The first place field recorded from the rat B2 was recorded in session 11 in the circular apparatus. In order to investigate if the cue card was a key feature in the rat's spatial orientation the cue card was rotated 180 °.
After that experiment the rat was tested in the square apparatus containing objects, and no place fields were detected.
The tetrodes were lowered further and in session 27 a place field was detected in the square apparatus containing objects.
The initial arrangement of the objects in the square apparatus was: the metal block, the glass sphere, the paper box and the small can of paint. The metal block was located at the upper left, the can of paint at the upper right, the paper box at the lower left and the glass sphere at the lower right.
The testing enclosure was changed between sessions in the following pattern (also see Figure 13): After the initial sessions the can of paint and the paper box were swapped, meaning the can of paint was positioned in the lower left corner. In the next session the rat was once more tested in the original object configuration, and then the can of paint was removed and replaced by the spool of metal wire. The rat was tested again in the original configuration before the recordings in the empty apparatus where reward was given started. The enclosure was polarised by a cue card located on the north wall and reward was sprinkled randomly across the area during the sessions. After the initial sessions the cue card was rotated 90 ° to the right, moving it to the east wall. The floor of the enclosure was wiped to remove any olfactory

cues. During the next recording the cue card was rotated back to the original position, and the final session was recorded using the apparatus containing objects in their initial configuration.

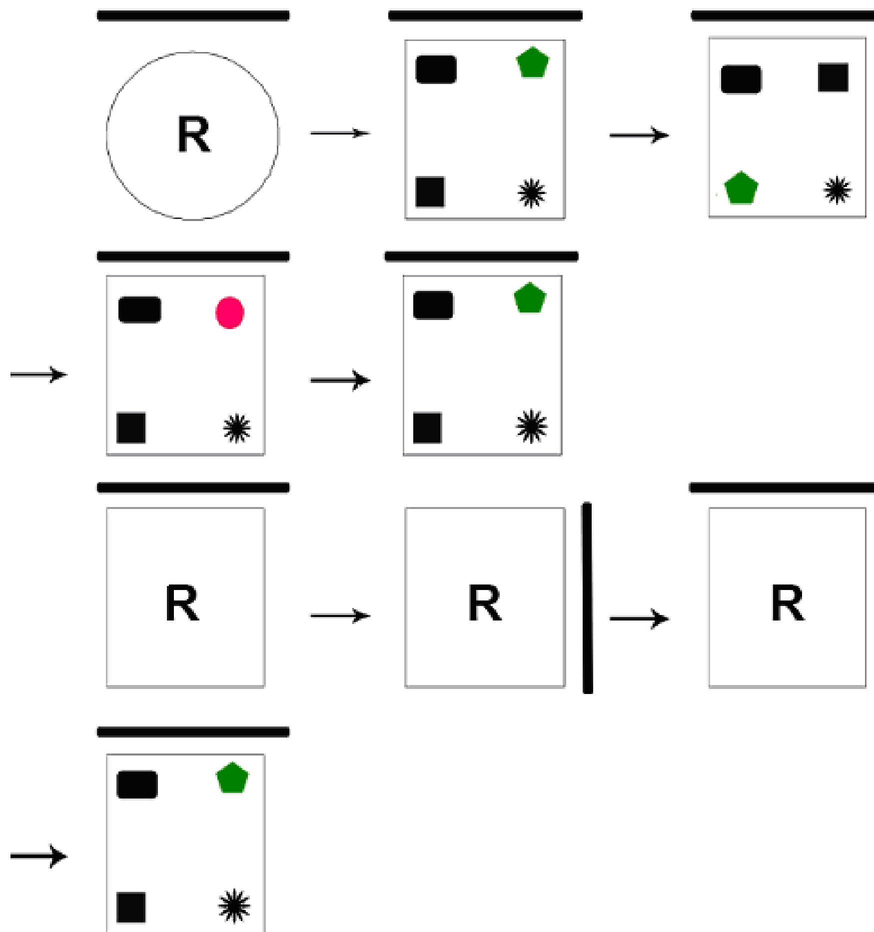



**Figure 13. The order in which the recording of sessions for rat B2 were done**. The altered configurations between sessions are also shown. The objects are represented by different symbols. The R shows apparatuses in which reward was given. The thick black line shows the position of the cue card. Symbols: The green pentagon represents the can of paint, the red circle the spool with metal wire, the star the glass sphere, the square the paper box and the rectangle the metal block.

Each of the configurations was used in at least two consecutive sessions. This was done to make it possible for place fields to develop and become stable. The exceptions were the sessions recorded in the square apparatus with the original object configuration before and after sessions where changes were made. However, at least three sessions were recorded in these enclosures, before any changes were made in the environment, to confirm that the cells and their firing fields were stable. The reason for testing in the same apparatus between sessions with different configurations was to see that the place field recorded previously was

still present and at the expected location. This would confirm that we had the same recoding conditions as far as tetrode positioning and recording equipment was concerned.
The reason the can of paint was used for our manipulations was that we saw a place field forming at its site. Therefore the following manipulations involved this object, to see what effect that had on the positioning and stability of the place field.

### Rat R3

R3 had very high cell activity, most of the time on all four tetrodes. At times so many spikes were recorded that clustering became difficult. Sessions were recorded in the circular apparatus, but no place specific activity was found. The rat was then tested in the square apparatus containing objects. No spatially modulated cells were found in this enclosure either. The rat was then tested in the square enclosure where it received reward to see if the presence of objects was necessary for the formation of firing fields.

## *Behaviour*

Rats are individuals, and some rats have an overall higher activity level and quickly become familiar with its new surroundings. Others are more careful and use a lot of time getting to know new surroundings, and some are more prone to grooming activity and spend a lot of time sitting still. Some general observations about how their surroundings influence their behaviour can still be made.
The rats had been handled and trained to explore a large area. Since they were used to being handled and being in unfamiliar environments their activity level in the first session in either apparatus was high. Objects present did however seem to keep the rats level of activity high for a longer period than in the empty apparatuses.

### Circular apparatus

The rats' first recording sessions were done in this apparatus. In the initial sessions they showed a pronounced tendency to keep close to the wall. This behaviour is called thigmotaxis. Reward was randomly dispersed in the box during the sessions, and the rats

quickly started traversing the area to collect the food. In the first session the food was often bought back to the wall before it was eaten; however the rats soon started eating the food where they found it.

The level of activity in this apparatus dropped quickly and in the following sessions the level of activity became more dependent on food pellets being given to encourage the rat to traverse the area.

### Square apparatus containing objects

In the first session in this enclosure the rats usually kept close to the walls at the beginning of the session, but soon made detours to where the objects were placed. In the following sessions it is apparent from the trajectory that the majority of the time was spent exploring the objects and less time was spent walking along the walls. The least amount of time was spent in the middle of the enclosure. The rats' movements in this apparatus, compared to the empty test apparatuses, cover more of the area. The trajectories show that the rats spent most of the time along the wall and close to the objects.

Some objects seemed to be subject to more intense exploration than others; this seemed to be the case for the can of paint. There was also an increased activity around the metal block. These two objects were also quite frequently urinated on by the rats.

After multiple sessions with the same objects, the activity level of the rat abated. The decrease in activity was most pronounced in the empty apparatuses. The substitution of the can of paint with the spool with metal wire caused a marked increase in the activity surrounding the new object in the first session containing this object, session 33. In session 34 the activity was only slightly higher in the area around the spool, which means the novel object was subject to exploration in the first session, though by the second session the activity level had returned to normal. The activity level also increased when the can of paint was returned to the enclosure.

### Square apparatus with reward

In the beginning the rats showed thigmotactic behaviour. After the first session they would start to leave the wall, particularly when food was dropped into the apparatus. Some rats would collect the food and then bring it back towards the wall before they ate it, like they did in the circular apparatus. This behaviour soon abated, and after a while the rats ate the food where they found it.

The trajectories show that the rats preferred to keep close to the wall, but during the course of a session the activity is quite evenly distributed in the apparatus. This is most likely due to the dispersing of food pellets. Thigmotaxis seemed to be more pronounced in the circular apparatus than in this square.

## *The data figures*

All the figures showing the recoded cell activity display three panels. The left panel in Figure 14, 15, 16, 17 and 20 and the second row from the top in Figure 18, 19 and 21 show the spike waveforms, with the shape of the first 100 spikes located on the left and the average spike waveform on the right. For the spike waveforms the x-axis is time (2 ms) and the y-axis spike amplitude (μV). The maximum spike amplitude is 280 μV. The middle panel in Figure 14, 15, 16, 17 and 20 and the third row in Figure 18, 19 and 21 show the cell's firing rate. The firing rate is indicated by a colour scale containing blue, green, yellow and red, blue signifying the lowest firing rate and red the highest. The right panel in Figure 14, 15, 16, 17 and 20 and the bottom row in Figure 18, 19 and 21 show the rats' trajectories (the rat's movement in space) superimposed by the firing of the selected spikes.
In the figures showing the results from the sessions recorded in the apparatus containing objects there is also a schematic presentation of the objects' position in the apparatus (Figure 18, 19 and 20).
The cue card is indicated by a black line. In the circular apparatus the direction is indicated by clock hours, in the square ones N, S, E and W indicate north, south east and west.
In each figure the rows or columns are marked with the corresponding session.

## *Place cell activity*

### Rat G2

Rat G2 was tested in the circular apparatus and in the square apparatus containing objects. After a couple of sessions in the square apparatus the cells that we had previously recorded disappeared. We raised the tetrodes, trying to relocate the signal. We then lowered the tetrodes further. The signal was however not recovered.

## Circular apparatus

In total 12 sessions were recorded in the circular apparatus. In session 6 a cell with spatially modulated firing was found. This cell was located on tetrode 1. Figure 14 shows the cell in session 6 and 7. In session 6 it has a firing field located at roughly 2 o'clock, in session 7 it has shifted slightly to 3 o'clock.
The spike waveforms suggest that the spikes originate from the same cell.

## Square apparatus containing objects

In total 6 sessions were recorded in this apparatus. Two cells with place specific activity were located in session 8.
The placement of the objects were the same as in the standard setup for rat B2, the metal object was located at the upper left side, the paper box at the lower left, the can of paint at the upper right and the glass sphere at the lower right.
The detected cells' place specific activity was stable in 3 sessions. Both figures (Figure 15 and 16) show results from session 8, 9 and 11. The cell activity was stable in session 10 as well, however session 9 and 10 were recorded with only minutes apart so we chose to omit the results recorded in session 9.
Cell #1 had a place field located in the upper right corner and cell #2 was active around the lower left object, the paper box.
Cell #1 was recorded on electrodes 1 and 3 of tetrode 4 (see Figure 15). In session 8 its firing field was found in the upper right corner. In session 9 and 11 the firing field had shifted slightly, and now seemed to have its focus close to the wall on the left side of the corner. The field stayed clearly defined during all three following sessions. The spike waveforms indicate that the spikes recorded in the three sessions most likely originate from the same cell.
Cell #2 was recorded by electrodes 1 and 3 of tetrode 1 and had a quite diffuse firing field in the first session, s8 (see Figure 16). It seemed to be active in two distinct areas, one surrounding the lower left object and one next to the middle of the upper wall. In the following two sessions (session 9 and 11) the firing field by the upper wall disappeared and the one by of the paper box became larger and more distinct.
The spike waveforms indicate that the spikes designated cell #1 and cell #2 originated from single cells.

### Rat B2

Rat B2 was the subject from which we got the most results. This rat was tested in all three enclosures, and changes in the environment were made in all three of them.
The tetrodes were not moved at any time during the testing period.

## Circular apparatus

In this apparatus the rat received reward during the period of gradual lowering of the tetrodes. 20 sessions were recorded in this apparatus. In session 11 a place field was detected and the lowering of the tetrodes stopped. The cell was located on all four electrodes of tetrode 2. Figure 17 shows the results from session 11, 12 and 19 in which a place field was located in the apparatus at a direction of approximately 10 o'clock. This field was stable in three consecutive sessions which are not shown here. In session 20 the cue card was rotated 180 °. The firing field did not follow to the cue card rotation; it remained in almost the same place, slightly more towards 12 o'clock than the previous sessions. The number of spikes recorded in this session compared to the previous session was much lower. It is difficult to determine whether the shape or size of the field changed during these sessions. This is mostly due to the fact that the rat did not traverse the entire enclosure.
The spike waveforms indicate that all the recorded spikes originated from the same cell.

## Square apparatus containing objects

A total of 19 sessions were recoded in the square apparatus containing objects.
In session 27 a cell with a place field was detected (see Figure 18). The cell was not the same cell that had been present in the circular apparatus; the new cell was located on a different tetrode. The activity here was recorded on all four electrodes of tetrode 3.
The cell that was active was numbered cell #1. Its place field was located in the upper right corner, close to the can of paint.
It had a stable place field in a total of three sessions before the object manipulations were done.

### *Relocating an object*

Previous to recording session 29 the positions of the can of paint and the paper box were exchanged, causing the can of paint to be located in the lower left corner. The cell's firing field also moved to the lower left corner, following the can of paint (see Figure 18), this

was also observed in session 30 and 31. In session 32 the can of paint was again returned to its original position, and the firing field also moved back. The firing field has the same shape and size in all the sessions. The shape of the spikes indicates that the spikes originate from the same cell.
The activity of the rat is not uniformly distributed across the apparatus. In session 27 and 29 the trajectory shows considerably higher activity in the parts of the environment containing objects. In session 27 the activity mainly surrounded the left side objects. In contrast it surrounded the upper objects in session 29, and in session 32 the rat's activity was mostly centred on the can of paint.
The cell with the firing field attached to the can of paint was active in a total of 10 sessions. It seemed to fire more frequently in the earlier sessions, the number of spikes and the size of the firing field decreasing throughout the testing period.

### *Replacement of an object*

In session 33 the can of paint was replaced by a spool of wire, the can of paint being the object cell #1 was attached to. Figure 19 shows the result of the replacement of the can of paint. The cell's firing field did not seem to change when the object was replaced. The size and shape of the firing field was approximately the same.
Two sessions were recorded with this object replacement, but only one is shown in the figure. From the recorded trajectories it is evident that the rat tended to spend most of the time in the area surrounding the can of paint, and when this object was removed the dwell time in this area seemed to be higher than in other areas of the apparatus.

## Empty square apparatus with reward

In session 37 the rat was moved to the empty square apparatus, where reward was dropped randomly during the session. 7 sessions were recorded.
Figure 20 shows the cells located in sessions 39, 40, 41 and 42 recorded in this enclosure. One cell with a firing field close to the west wall was found. The firing field was a bit diffuse. This cell was numbered cell #2.
In session 40 and 41 the cue card was rotated 90 ° to the right. The firing field shifted to a position close to the south wall. When the cue card was rotated back to its original position the firing field also returned, however it seemed to have moved slightly towards the south. It was also far less distinct, and the cell seemed to be less active.

After the last session in the square apparatus where reward was given the rat was retested in this apparatus containing objects. Three sessions were recorded. One of these sessions, session 46 is shown in Figure 21.
Figure 21 shows cell #1 recorded in session 27 with a clearly defined firing field attached to the can of paint. It also shows the cell recorded in session 46 found to have a firing field in the upper left corner, by the metal block placed here. By comparing the spike waveforms shown in this figure it is seems unlikely that the spikes recorded in session 27 and session 46 originated from the same cell.

## Differentiating the registered cells

Figure 22 shows the spike waveforms for 9 sessions recorded during the testing period for rat B2. It shows that from session 27 to 36 the signal on electrode 2 decreased slightly, whereas the signal on electrode 3 and 4 increased. The signal on electrode 1 remained the same. Between sessions 36 and 39 the signal changed drastically, most strikingly the spikes on electrode 1 and 2 disappeared. The recordings in the square apparatus containing objects stopped after session 36, in session 39 the empty square apparatus was used.
These observations suggest that the change from the no-reward to the reward condition caused a remapping. Cell #1, which was active in the apparatus containing objects, became inactive in the empty apparatus, and cell #2, which was not found in the sessions recorded in the apparatus containing objects, became active.
The spike waveforms originating from the sessions 39-46 did not vary much. The implication of this might be that the spikes in these sessions originated from the same cell (cell #2). Since session 39-43 were carried out in the empty apparatus and session 46 in the apparatus containing objects the uniform waveforms are interesting. The observations suggest that the cell active in session 46 is the same cell that was active in the empty apparatus (cell #2), not the one previously active in the apparatus containing objects (cell #1).
This means that cell #2, remapped when the rat was moved from the empty apparatus to the one containing objects. In session 39-42 its firing fields were placed close to the walls of the enclosure (Figure 20), in session 46 it had a clearly defined firing field surrounding the upper left object (Figure 21).

### Rat R3

R3 had a large number of active cells with high firing frequencies and high amplitude. These cells appeared a few testing sessions following implantation. Analysing the results was difficult due to the large amount of spikes recorded. In the circular apparatus (23 sessions), in the empty square apparatus (16 sessions) and in the apparatus containing objects (16 sessions) a high level of cell activity was recorded. Spatially modulated cells were found in neither of the apparatuses.
Since no spatially modulated cells were found the tetrodes were lowered between sessions. Figure 23 shows the clusters cut from a session 28 which was recorded in this apparatus.
It shows the high number of spikes recorded and the spike waveforms of cluster 1, 3 and 5. Cluster 1 contains 7369 spikes, cluster 3 6588 and cluster 5 235.
The waveforms of the spikes have shapes characteristic of neuronal firing, which means the spikes probably do originate from a neuron.

## *Autocorrelation results*

To confirm that the isolated spikes do originate from single cells autocorrelation was performed.
For the two cells isolated from rat B2 and the two cells isolated from rat G2 autocorrelation was performed to confirm that the spikes originated from single cells. Figure 24 shows the graphic representation of autocorrelations from two sessions with rat B2 and one with rat G2. The clusters that have been correlated stems from, for G2, session 9, and for B2 from sessions 28 and 40 where cell #1 and #2 were found, respectively.
From the figure it is apparent that for all four cells the inter spike interval is more than 2 ms between the majority of the spikes, thus suggesting that they are derived from the same cell.

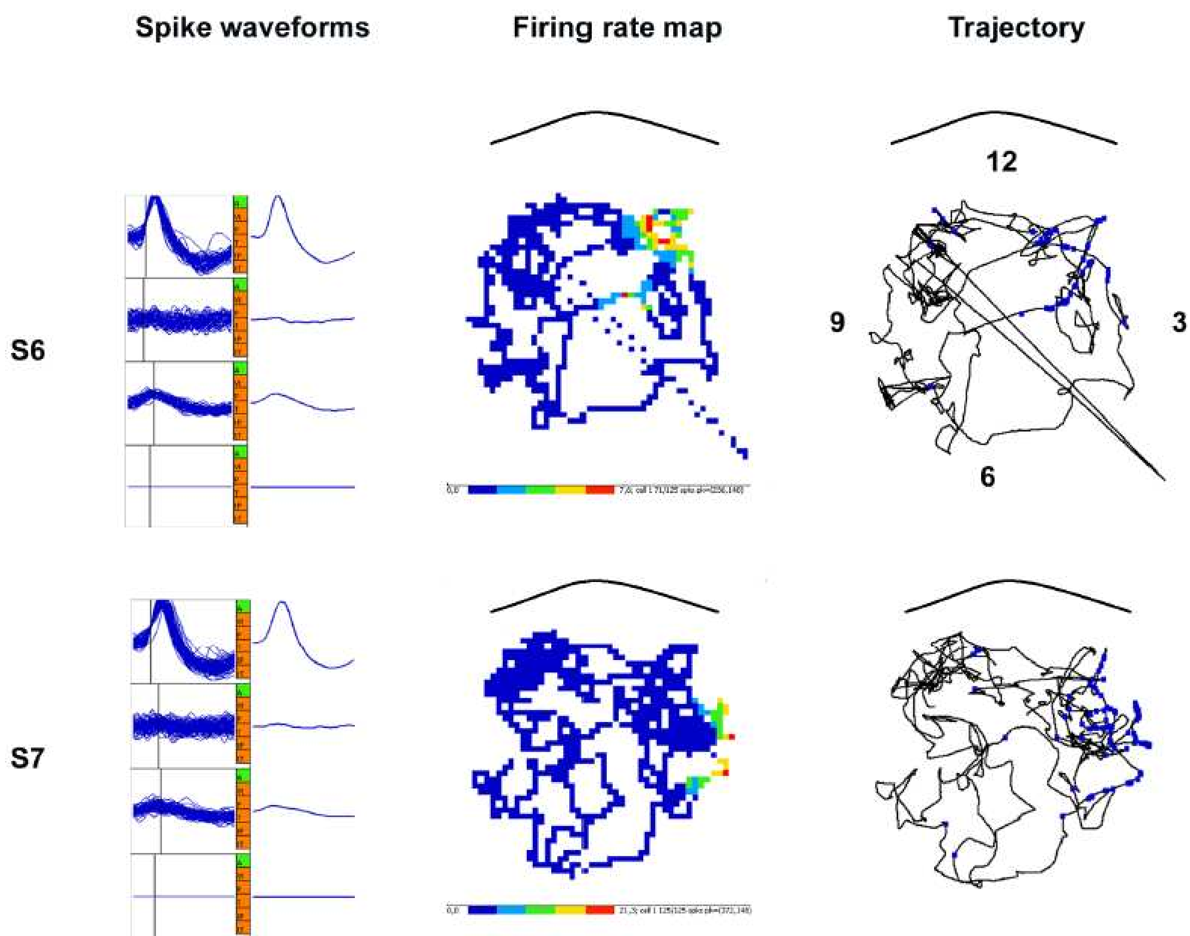



**Figure 14. The cell activity in the circular apparatus with reward in session 6 and 7 for rat G2**. The black line in firing rate map and the trajectory indicates cue card position. Spike waveforms from tetrode #1; x-axis 2 ms and y-axis 280 µV. The numbers in the trajectory map indicates position in hours.

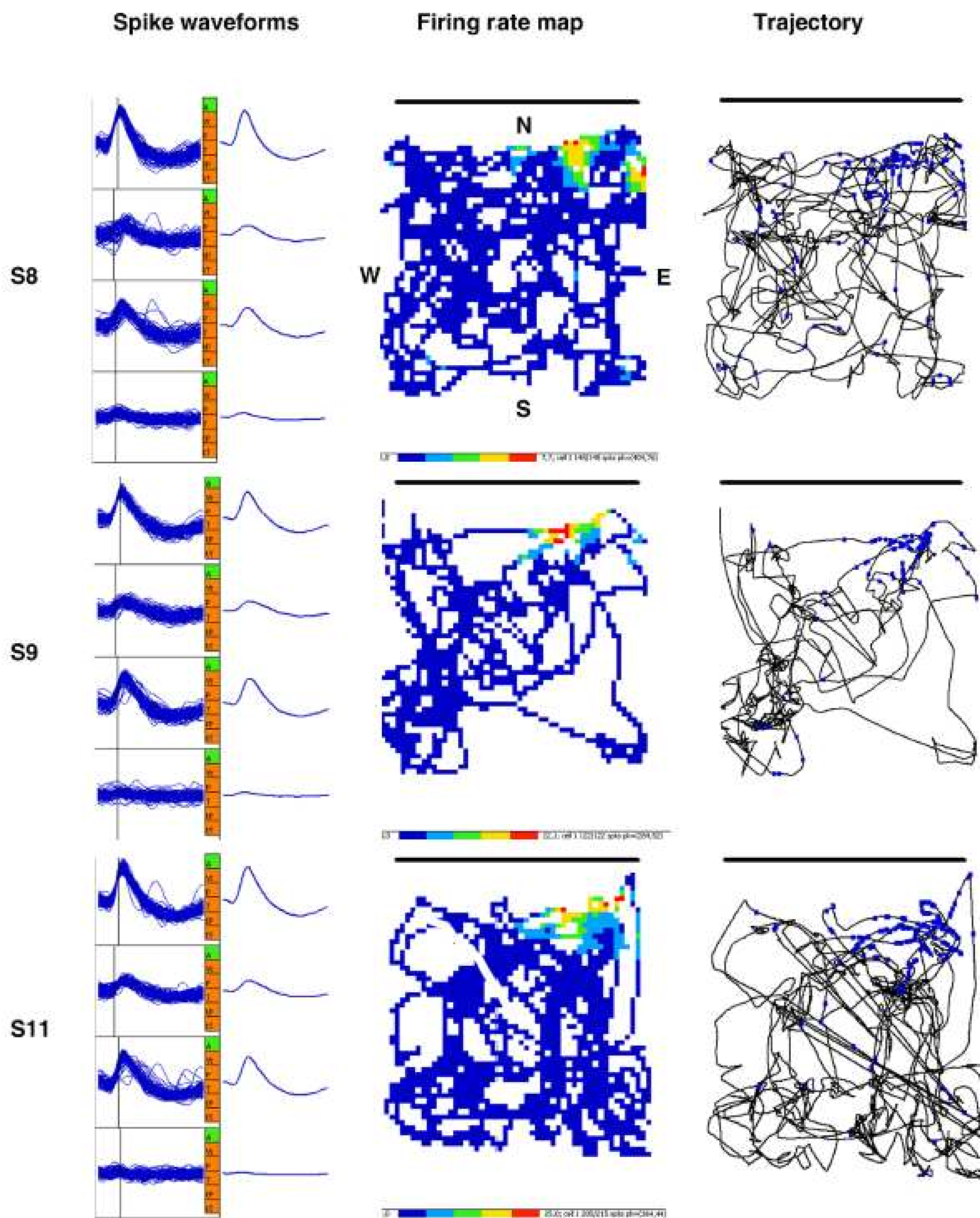



**Figure 15. The activity of cell #1 recorded in the square apparatus containing objects in sessions 8, 9 and 11 for rat G2**. Black line in firing rate map and the trajectory indicates cue card position. Spike waveforms from tetrode #4; x-axis 2 ms and y-axis 280 μV. The letters in the firing rate map indicates direction.

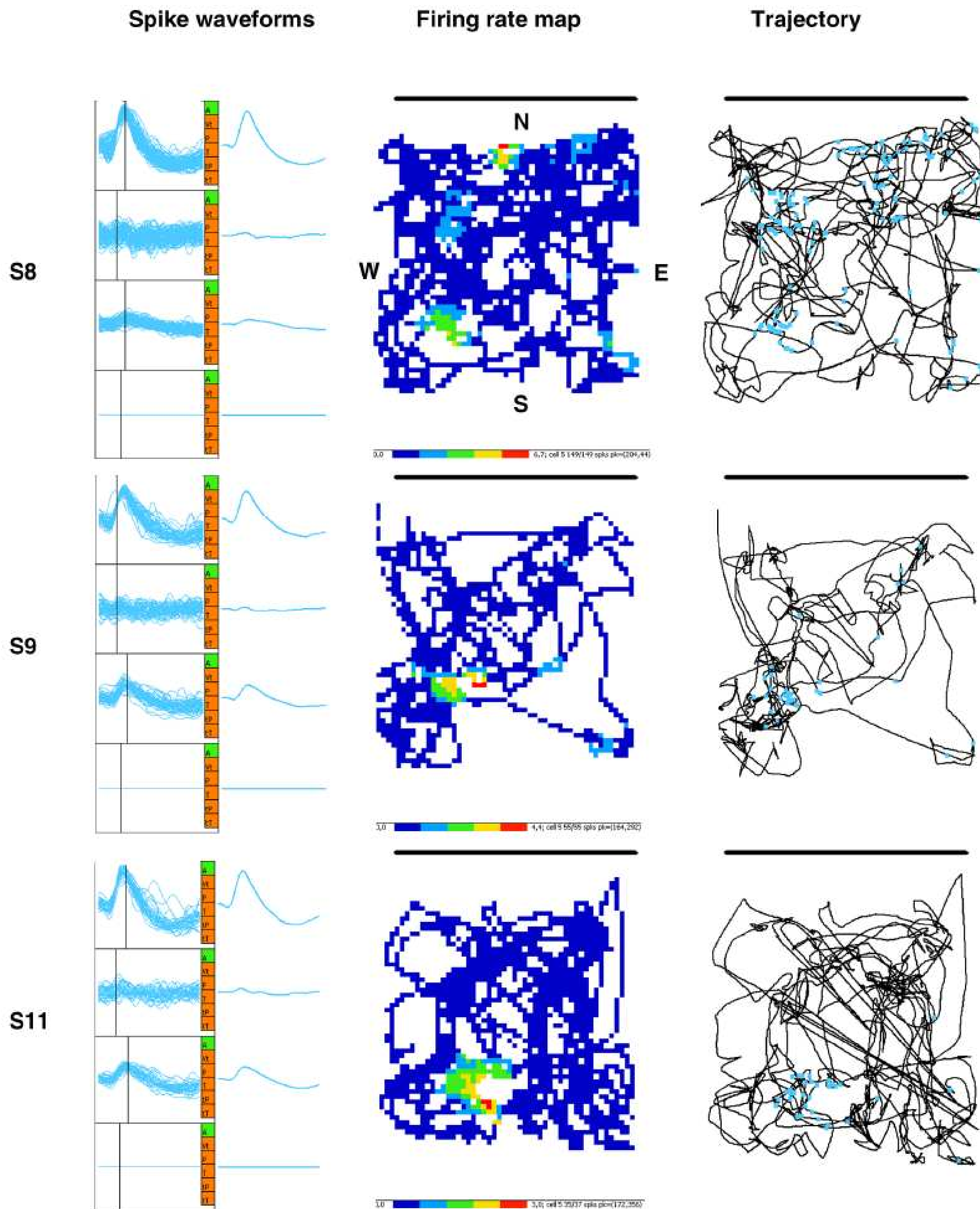



**Figure 16. The activity of cell #2 recorded in the square apparatus containing objects in sessions 8, 9 and 11 for rat G2.** Black line in firing rate map and the trajectory indicates cue card position. Spike waveforms from tetrode #1; x-axis 2 ms and y-axis 280 μV. The letters in the firing rate map indicates direction.

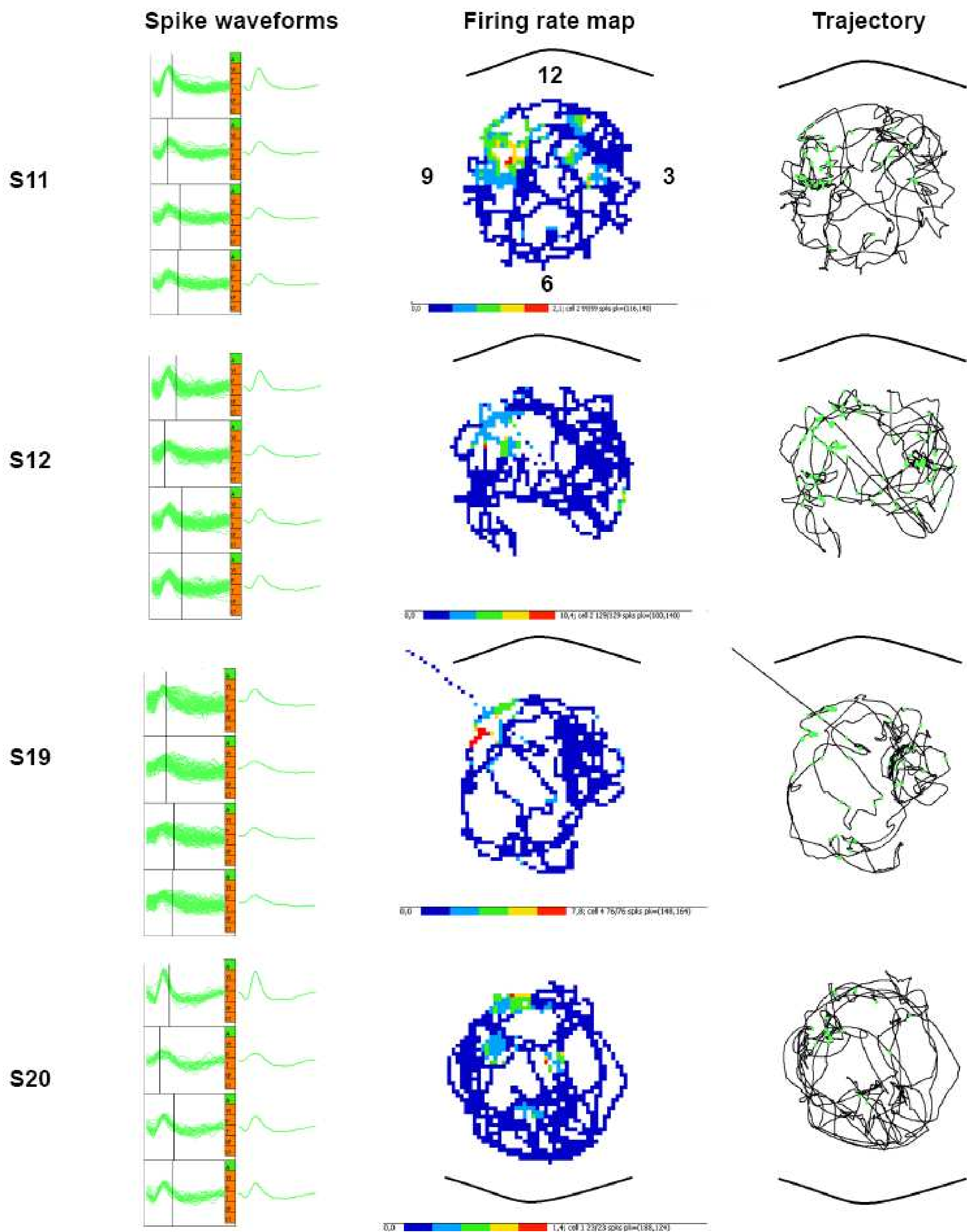



**Figure 17. The cell activity in the circular apparatus with reward in sessions 11, 12, 19 and 20 for rat B2.** Black line in firing rate map and the trajectory indicates cue card position. In session 20 the cue card was rotated 180 °. Note the decrease in spike number apparent in the spike waveform panel for session 20. Spike waveforms from tetrode #2; x-axis 2 ms and y-axis 280 µV. The numbers in the firing rate map indicates position in hours.

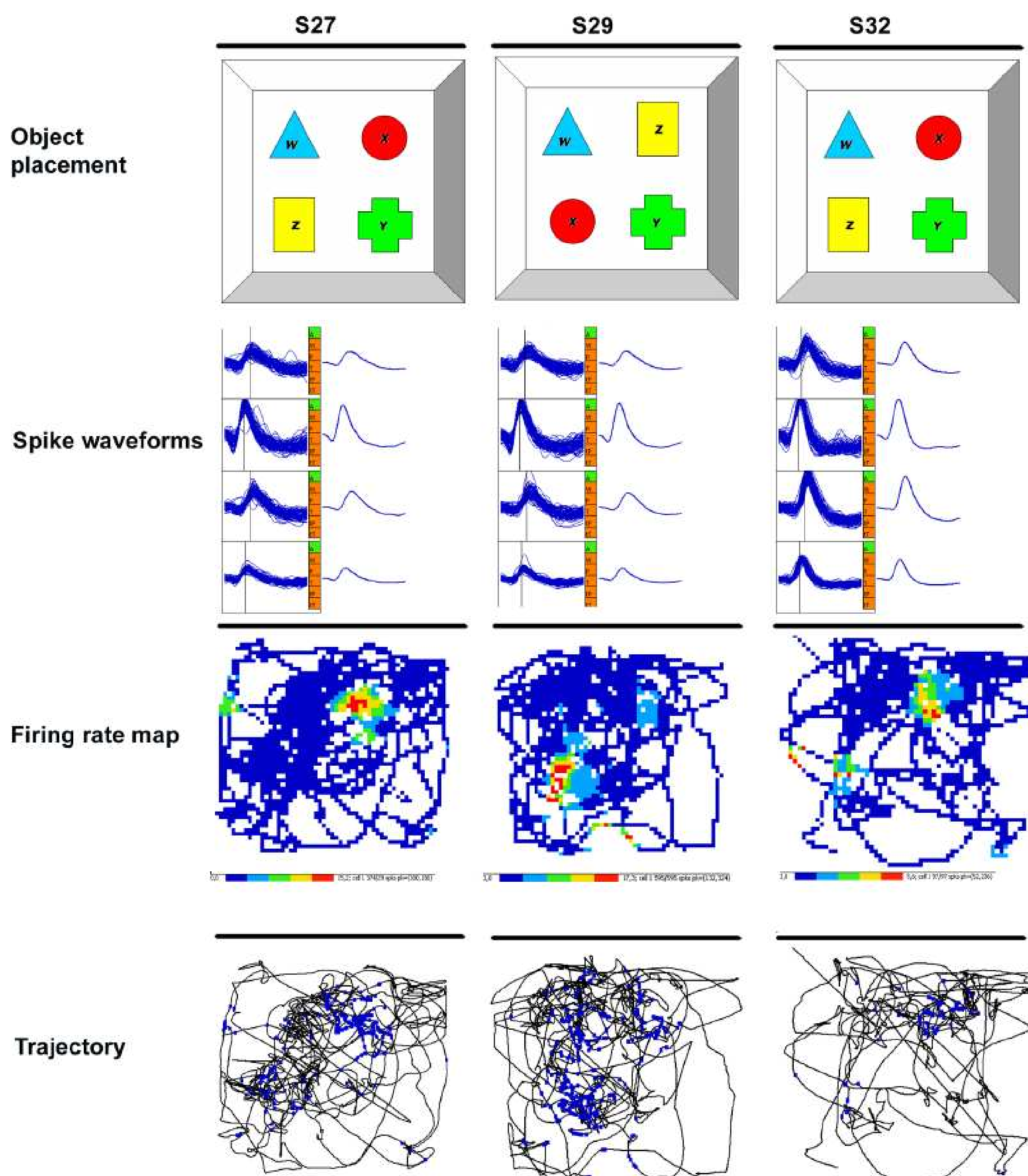



**Figure 18. The cell activity in the square apparatus containing objects in sessions 27, 29 and 32 for rat B2.** The upper row shows the object placement, the objects being: X- can of paint, W- metal block, Z- paper box and Y- glass sphere. In session 29 the positions of the can of paint (X) and the paper box (Z) were exchanged. The black line in the Object placement, Firing rate map and Trajectory indicates the cue card placement. Spike waveforms for tetrode #3; x-axis 2 ms, y-axis 280 µV.

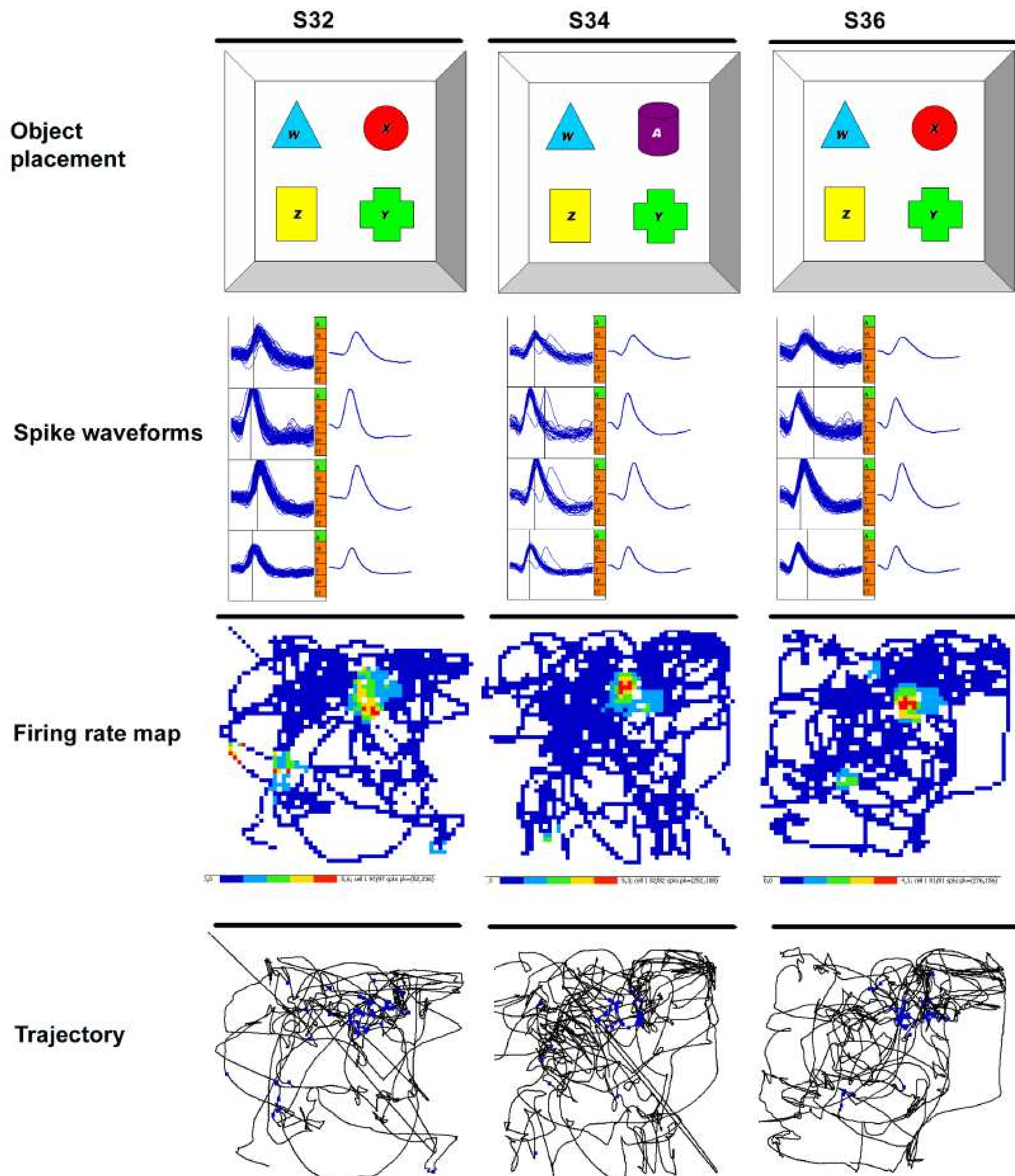



**Figure 19. The cell activity in the square apparatus containing objects in session 32, 34 and 36 for rat B2.** The upper row shows the object placement, the objects being: X- can of paint, W- metal block, Z- paper box, Y- glass sphere and A- spool with metal wire. In session 34 the can of paint (X) was replaced by a spool with metal wire (A). The black line in the Object placement, Firing rate map and Trajectory indicates the cue card placement. Spike waveforms for tetrode #3; x-axis 2 ms, y-axis 280 µV.

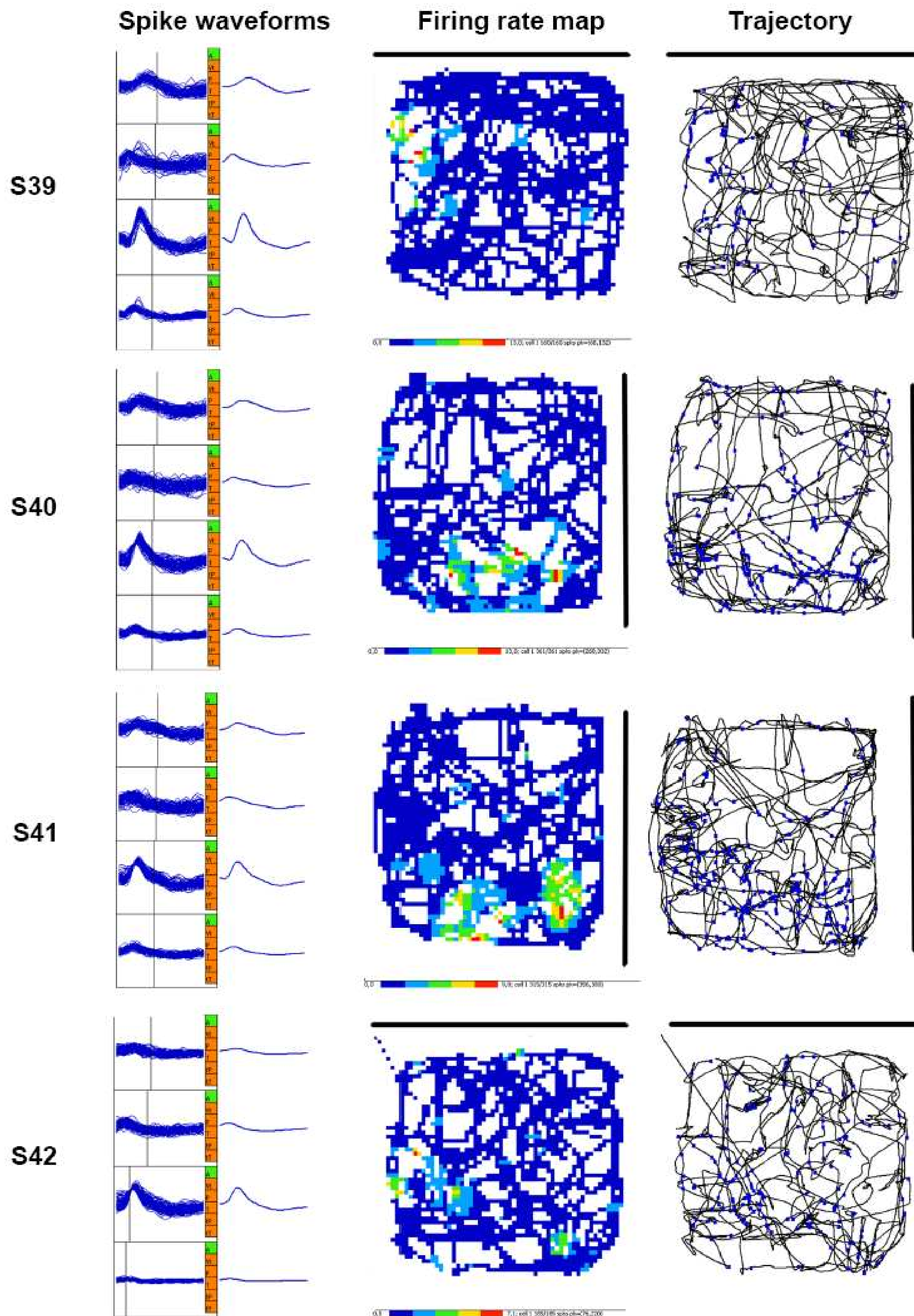



**Figure 20. The cell activity in the empty square apparatus where the rat received reward in session 39, 40, 41 and 42 for rat B2.** The black line in the Firing rate map and Trajectory indicates the cue card placement. In session 41 the cue card was rotated 90 °. In session 40 and 41 the cue card was rotated 90° to the right. Spike waveforms for tetrode #3; x-axis 2 ms, y-axis 280 µV.

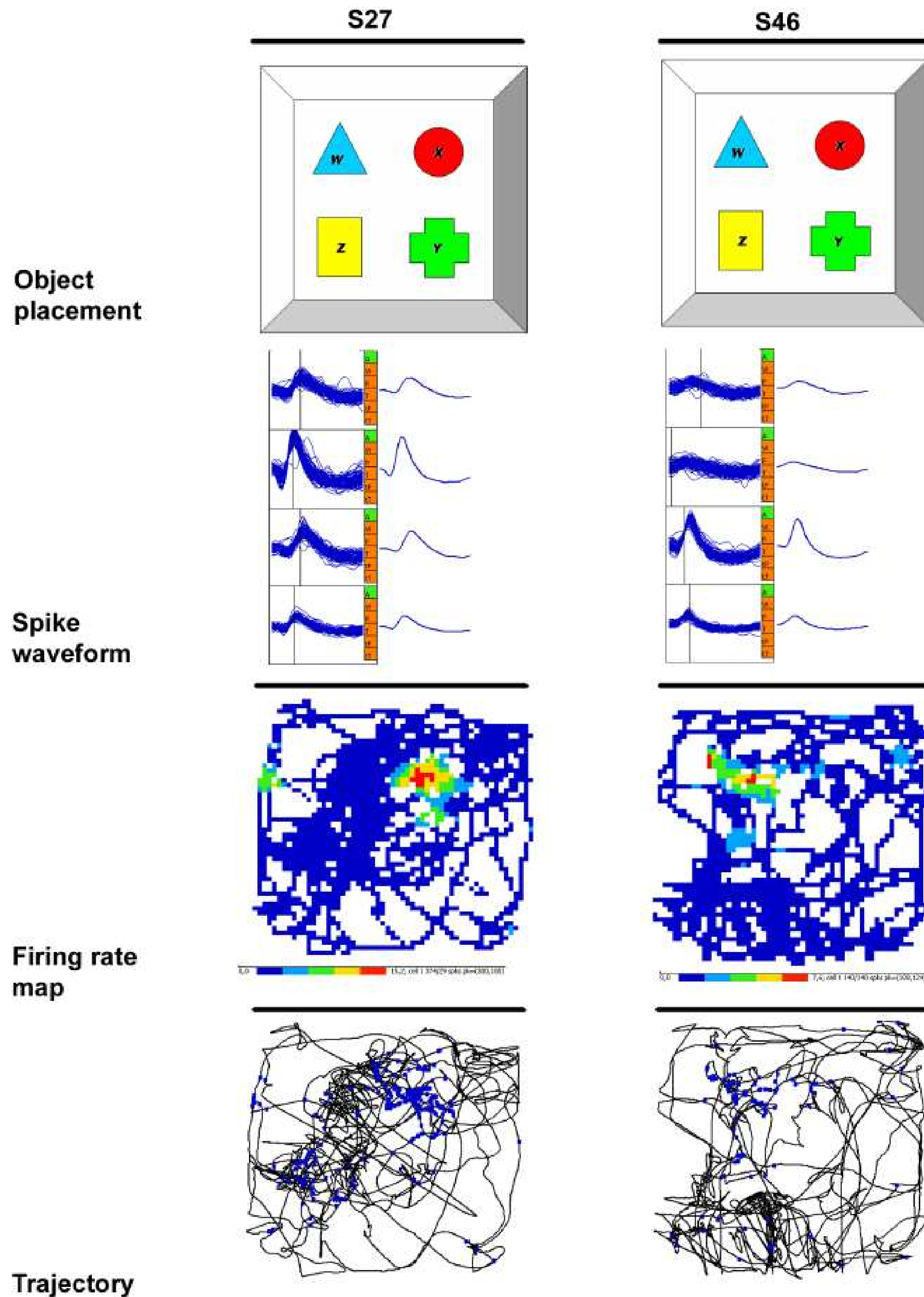



**Figure 21. The cell activity in the square box containing objects in sessions 27 and 46.** Session 27 was the first session in which spatially modulated activity was found in the square apparatus containing objects, session 46 was the last session recorded in this apparatus. In between these sessions the sessions in the empty square apparatus was performed. The black line in the Object placement, Firing rate map and Trajectory indicates the cue card placement. Note the change in spike waveform from session 27 to 46. Spike waveforms from tetrode #3; x-axis 2 ms, y-axis 280 µV.

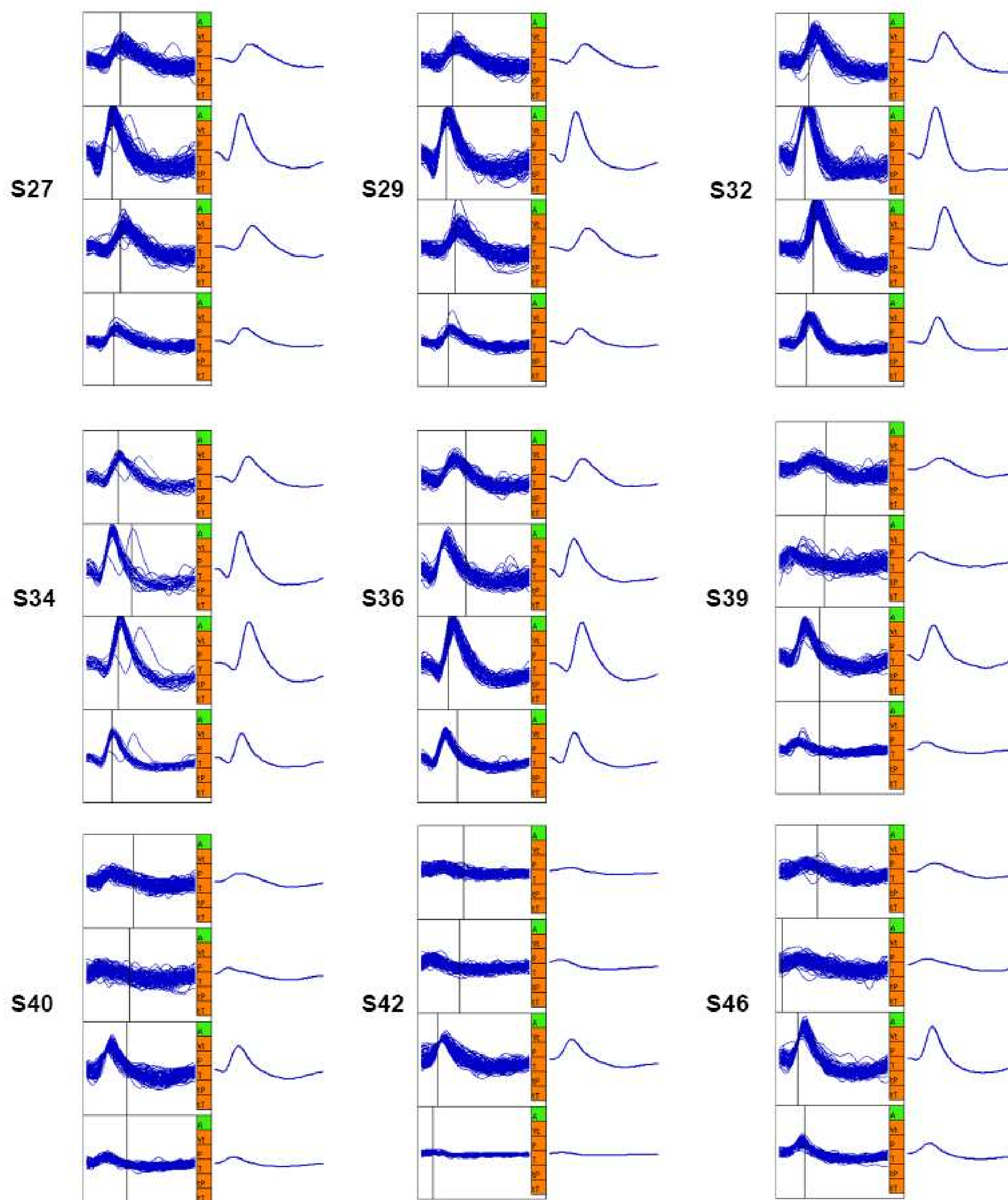



**Figure 22. The spike waveforms for the cells recorded with tetrode #3 in sessions 27, 29, 32, 34, 36, 39, 40, 42 and 46 for rat B2.** Sessions 27, 29, 34, 36 and 46 were recorded the square box containing objects. Sessions 39, 40 and 42 were recorded in the empty square box where the rat received reward. The cells' firing fields can be seen in previous figures. Notice the shift in shape of the waveforms between session 36 and 39. X-axis 2 ms and y-axis 280 µV.

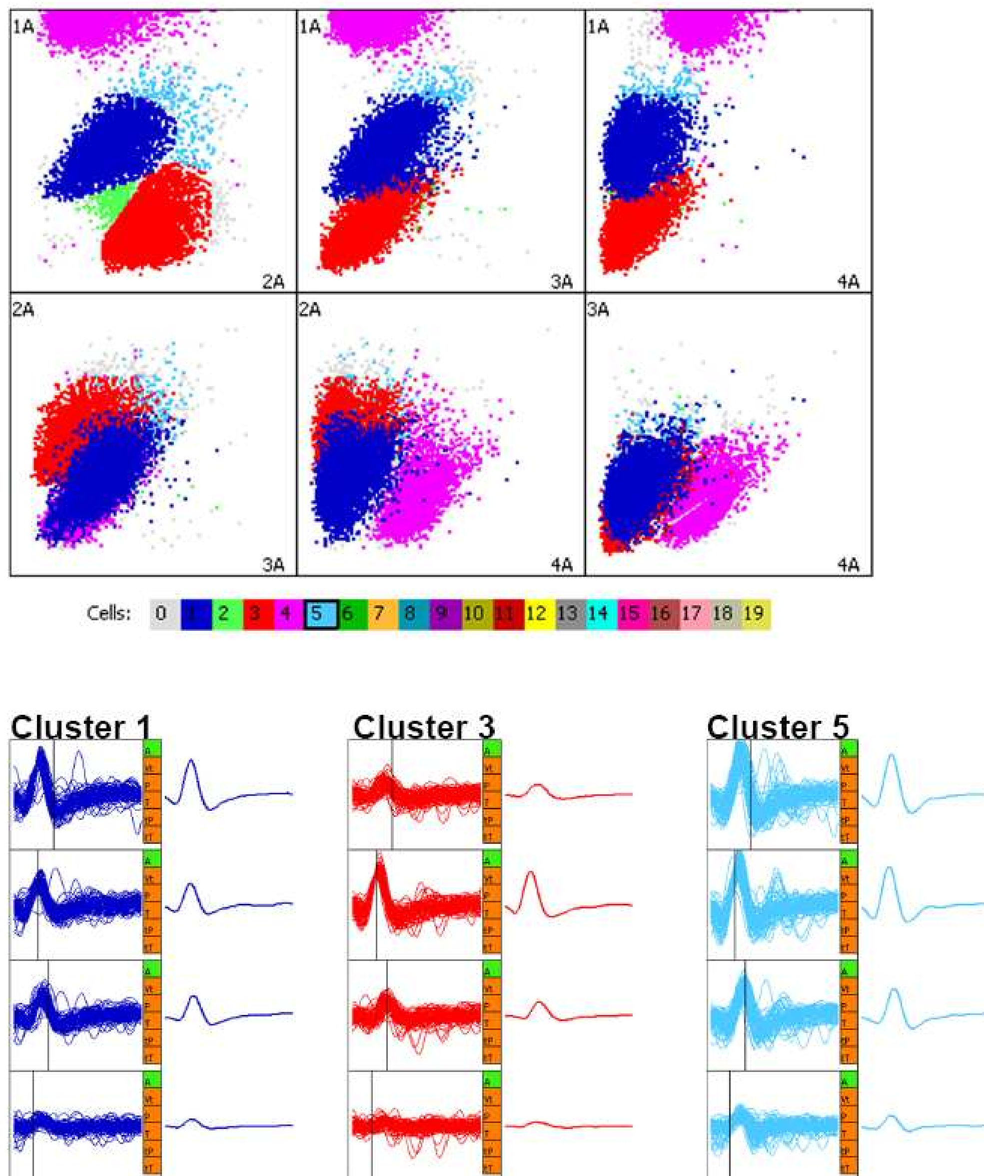
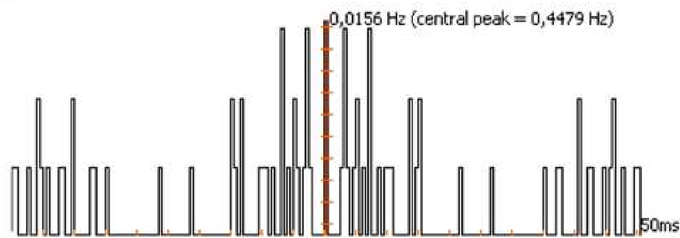
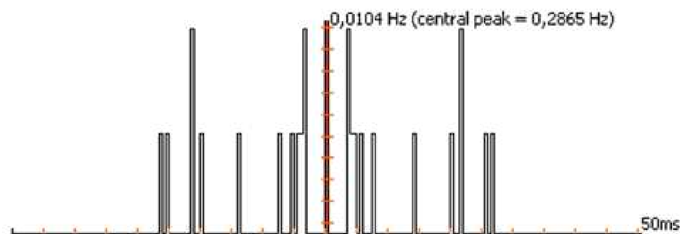



**Figure 23. The cell activity in a session registered from rat R3.**The activity was registered in session 28 from tetrode 4 in the empty square apparatus where the rat received a reward. The upper part of the figure shows the cluster cutting window in Tint Cluster Cutting and Analysis Software. Five clusters have been found and colour marked. The bottom part shows the spike waveforms of three of the clusters found in the upper picture; cluster 1, 3 and 5. Cluster 1 contained 7369 spikes, cluster 3 6588 and cluster 5 235 spikes.  Spike waveforms; x-axis 2 ms, y-axis 280 µV.

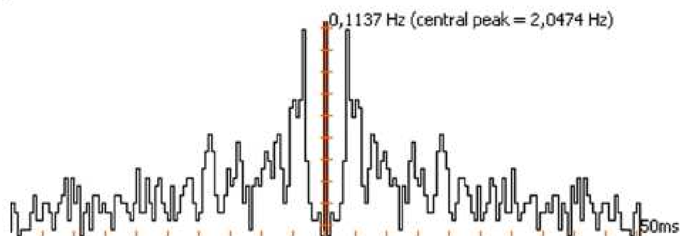
**G2**
**Cell 1**




**G2**
**Cell 2**




**B2**
**Cell 1**




**B2**
**Cell 2**

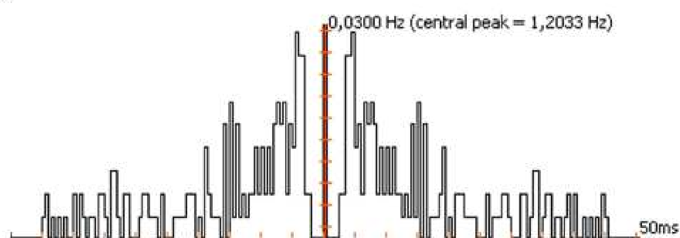



**Figure 24. Autocorrelograms for the firing frequency of the cells recorded in rat G2 and B2 in the square apparatuses.** The autocorrelations of G2 cell #1 cell #2 were done from session 9, for B2 the autocorrelation of cell #1 was done from session 28, and cell #2 from session 40. The difference in shape of the figures from rat G2 and B2 is caused by the higher number of spikes recorded from cell #1 and #2 in rat B2 than in the sessions with rat G2. The spike number for G2 in session 9 was cell #1: 184 and cell #2: 55. For B2 the spike number in session 28 for cell #1 was 432 and for cell #2 in session 40 it was 361.
X-axis time (ms) ranging from -50 ms to + 50ms in reference to the time of spike occurrence, y-axis frequency (Hz).

## *The firing rates of the isolated complex spike cells*

A way for place cells to encode for changes in their environment is by rate remapping. In this study we calculated the overall firing rate which indicates the average activity level of the cell throughout the session.

For all the sessions where cells with stable firing fields were found the overall firing rate of the cell was calculated by dividing the number of spikes recorded presumed to originate from the cell in question by the duration of the session (in seconds).

| Rat B2 Cell #1 | Session number | Firing rate (Hz) |
|---|---|---|
| Square w/objects Standard | 27 | 1.25 |
| | 28 | 2.05 |
| Square w/objects Paint can swapped place | 29 | 1.96 |
| | 30 | 0.63 |
| | 31 | 0.49 |
| Objects Standard | 32 | 0.44 |
| Square w/objects Paint can replaced | 33 | 0.46 |
| | 34 | 0.27 |
| Objects Standard | 35 | 0.27 |
| | 36 | 0.27 |

**Table 2. The overall firing rate for cell #1 recorded from rat B2 during the sessions the square testing apparatus containing objects.** The firing rate was found by dividing the number of spikes isolated as cell #1 by the length of the session.

Cell #1 in rat B2 shows a gradual decrease in firing rate during the testing period.

| Rat B2 Cell #2 | Session number | Firing rate (Hz) |
|---|---|---|
| Empty square, reward given | 37 | 0.56 |
| | 38 | 0.46 |
| | 39 | 0.53 |
| Empty square, cue card rotated 90° | 40 | 1.2 |
| | 41 | 1.05 |
| Empty square, reward given | 42 | 0.63 |
| | 43 | 0.24 |
| Square w/objects Standard | 44 | 0.45 |
| | 45 | 1.46 |
| | 46 | 0.49 |

**Table 3. The overall firing rate for cell #2 recorded from rat B2 during sessions recorded in the square apparatuses**. The firing rate was found by dividing the number of spikes isolated as cell #2 by the length of the session. Note the increase in firing rate in session 40, 41 and 45.

Cell #2 in rat B2 shows an increase in firing rate when the cue card was rotated. The firing rate overall in the other sessions is relatively stable, the exception being session 45.

| Rat G2 Cell #1 | Session number | Firing rate (Hz) |
|---|---|---|
| Square apparatus w/objects Standard | 8 | 0.55 |
| | 9 | 0.96 |
| | 11 | 0.70 |

**Tabell 4. The overall firing rate for cell #1 recorded from rat G2 during the sessions recorded in the square apparatus containing objects.** The firing rate was found by dividing the number of spikes isolated as cell #1 by the length of the session.

| Rat G2 Cell #2 | Session number | Firing rate (Hz) |
|---|---|---|
| Square apparatus w/objects Standard | 8 | 0.82 |
| | 9 | 0.29 |
| | 11 | 0.12 |

**Tabell 5. The overall firing rate for cell #2 recorded from rat G2 during the sessions recorded in the square apparatus containing objects.** The firing rate was found by dividing the number of spikes isolated as cell #2 by the length of the session.

Cell #1 found in rat G2 seemed to have a relatively stable firing rate. Cell #2 showed a rapidly decreasing firing rate. It is difficult to say if there is an actual decrease in firing rate variations due to the fact that only three sessions were recorded before the cell activity disappeared.

## *Histology results*

After the experiments were ended most rats were put to sleep and prefused with formaldehyde or performaldehyde. The brain was dissected out of the skull and the brain put in formaldehyde and later sucrose- solution. Slices were made and stained using Cresyl Violet. The underside of the skull where the tetrodes protruded was examined.

### Rat B2

The implant was examined and the tetrodes protruding from the base of the skull found to in a tight bundle. The tetrodes seemed to be angled backwards. The approximate lengths of the tetrodes were measured to be 2.8-2.9 mm. The calculated depth was 2.15 mm.
Figure 25 shows the microdrive cemented on top of the skull and the prefused rat brain. The T indicates the tetrodes protruding from the base of the skull, and the G the ground screw. On the cortex of the brain the C marks the indentation made by the outer cannula of the tetrodes. Indentations caused by the ground screw and the screws used to secure the implant to the skull can also be seen on the surface of the cortex.

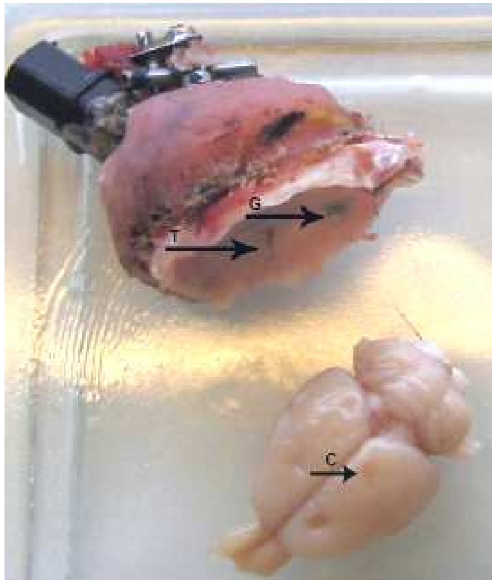



**Figure 25. The brain and implant of rat B2 after dissection.** The arrow marked T indicates the tetrodes protruding from the base of the skull. G indicates the ground screw and the C indicates the indentation made in the skull by the cannulas where the tetrodes were lowered into the brain. The other indentations seen on the surface of the cortex are made by the ground screw and screws used to fasten the implant to the skull.

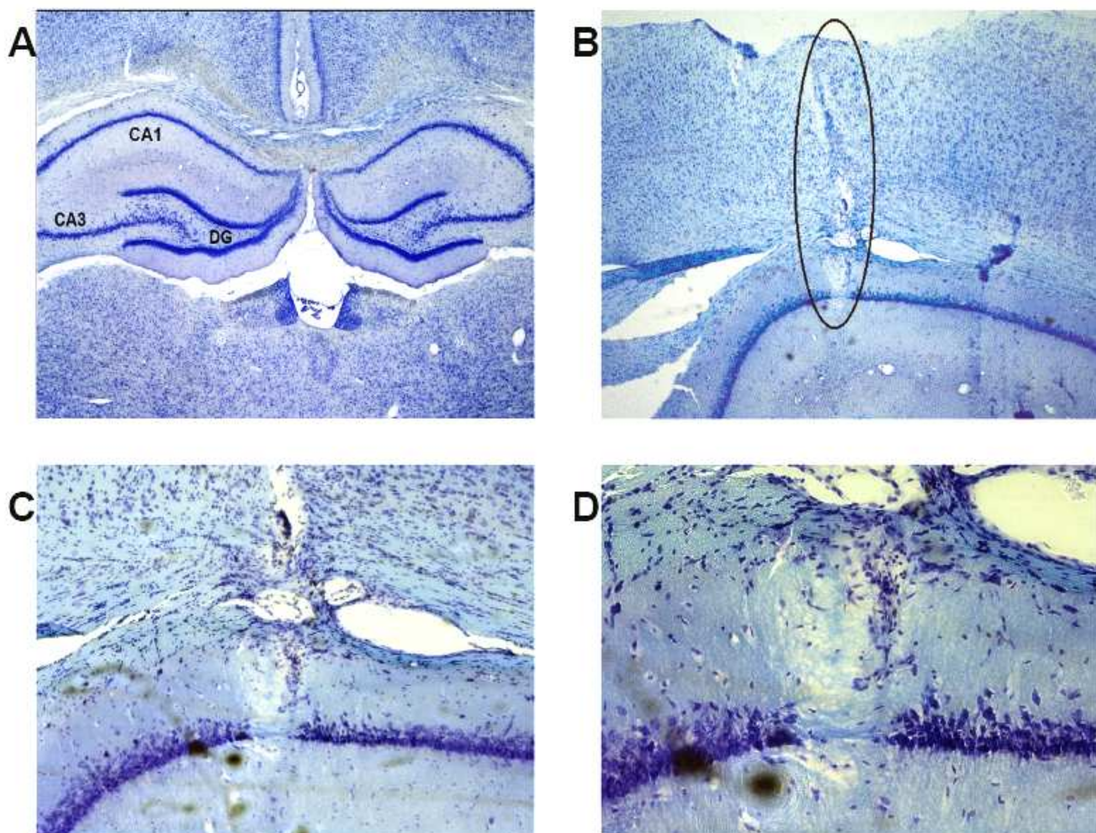



**Figure 26. Histology slides of the hippocampus, showing the path of the tetrodes in rat B2's hippocampus.** Picture A shows a frontal section of the hippocampus, the CA1, CA3 and dentate gyrus (DG) is indicated. The slide was made from the brain of rat R2. The next three pictures show slides from the hippocampus of rat B2 with increasing magnification. Picture B shows a section of the cortex above the right hippocampus with the tetrode track encircled. Picture C and D show the CA1-area where the tetrodes hit the CA1- cell layer. Note how the cell layer of CA1 has been disrupted by the tetrodes.

Figure 26 shows the frontal sections of the rat B2's hippocampus with an increasing magnification. In the upper right section the tetrode track is visible and in the two lower sections the end of the tetrode track can be seen, the disruption of the CA1 layer by the tetrodes is clearly visible.
The position of the tetrodes indicates that the registered cell activity originated in the CA1-layer of the hippocampus.

### Rat G2

When this rat had completed testing it was put to sleep, but it was not perfused, and no histology was obtained from the brain. Visual inspection of the brain showed that the tetrodes were in a tight bundle, but were slightly bent towards the back of the brain. The lengths of the tetrodes were measured to approximately 2.6 mm. The calculated depth was 2.35 mm.

## *Summary of the results*

### Rat G2

- One spatially modulated cell was found in the circular apparatus, with a distinct firing field located in the direction 2- 3 o'clock, shown in Figure 14.
- Two cells with distinct firing fields were present in the square apparatus containing objects. Figure 15 and 16. Cell #1 had a firing field located in the upper right corner, and cell #2's field was located around the lower left object, the paper box.
- For unknown reasons all the cell activity disappeared from the recordings.

### Rat B2

- A cell with spatially modulated firing was located in the circular apparatus. This cell's firing field did not follow the rotation of the cue card, it stayed in place. See Figure 17.
- A spatially modulated cell was found in the apparatus containing objects. Cell #1 had a firing field that was located around the can of paint. This firing field followed the can of paint when it was moved to a new location, but stayed in place when the can of paint was replaced by another object. See Figure 18 and 19.

- Another spatially modulated cell was found in the empty square apparatus. Cell #2 had a firing field located by the west wall. This field did not follow the rotation of the cue card, in stead a new firing field developed. See Figure 20.
- By histological analysis the tetrode track was localised going from the cortex to the CA1-area. The track stopped in the CA1-area causing damage to the cell layer, see Figure 26. This indicates that the cells recorded here were located in the CA1-area of the hippocampus.

### Rat R3

- Cell activity was found in the circular apparatus and both the square apparatuses. Many cells fired with a high frequency, however no spatial modulation was found. The number of high frequency cells made it difficult to isolate cells with a lower firing frequency and possible spatial modulation difficult.

# Discussion

The first part of this section contains the discussion of the results, both behaviour and place field development and the effects of changes in apparatus and manipulations of its contents.
I will then try to highlight some issues concerning our results and finally the implications of our findings.
This study reports results that were obtained from two of the 15 implanted rats. The second part of this section presents possible explanations for our low success rate in finding complex spike activity.

## *Experimental subjects*

The rat brain has been thoroughly mapped out over the years, the anatomy of the brain and many specific structures are therefore known. Many of the brain structures, including the hippocampus, have been described extensively. Rat behaviour as well as its relation to the function of specific structures has also been studied.
The rat hippocampus is a quite large structure which makes it, as well as the different areas of it, easy to locate. This makes it a good research model.
The basic hippocampal architecture is similar in the rat and human brain, there are some differences, but the basic structure of the cell layers and the connectivity is comparable. Animal studies of the hippocampus have therefore been valuable to understanding the human brain [10].
We used male Long Evans rats in this study because this strain has good hearing, good eyesight and is easy to handle.

## *Discussion of results*

### Behaviour

Thigmotactic behaviour was observed in all three apparatuses. Thigmotaxis describes the movement pattern in which the rat prefers to walk along the solid parts of an environment, in our case the walls or the objects. In this study movement along the walls seemed to be more pronounced in the circular apparatus than in the other two. That less movement along the walls was observed in the apparatus containing object was most likely explained by the

presence of objects and the rats' exploration of them. It was less expected that there should be a difference in thigmotactic behaviour between the two square apparatuses. There can be several possible reasons for this difference in movement pattern. One possible explanation might that the first sessions were recorded in the circular apparatus, and that the rats had become more accustomed to the testing procedure when sessions in the square apparatus were recorded. Difference in behaviour could also be due to variation in the amount of food that was given during the sessions. The unsystematic scattering of food pellets were not done after any predetermined pattern and it might be that the experimenter distributing the pellets in the empty square apparatus provided the rat with more food than in the circular apparatus. If more food was available in a session than in another the amount of time the rat spent away from the walls of the enclosure would increase.

In the apparatus containing objects the rats' movement patterns were evenly distributed between walking along the walls and exploring the objects. The activity was however not as evenly distributed among the separate objects. Which objects the activity centred around differed between sessions. The overall activity level seemed to be higher around certain objects, particularly the metal block and the can of paint. The fact that these two were the only metal objects is probably insignificant.
For rat B2 exchanging the can of paint with a novel object caused a marked increase in the activity level, particularly surrounding the novel object in the very first session this object was present in. The activity pattern returned to normal in the following session.
Similar results were reported by Lenck-Santini et al. they found that substituting an object with a novel one causes a significant increase in activity level, particularly surrounding the novel object [45].

### The firing patterns of the place cells

Both for rat G2 and B2 stable place fields were found in the circular apparatus and in the apparatus containing objects. In addition rat B2 had a cell with a place field located in the empty square apparatus.
The stability of these place fields was confirmed through several sessions, conducted in the course of at least two consecutive days.

For rat B2 cell #1 was located in the apparatus containing objects, and cell #2 was located in the empty square apparatus. In addition cell #2 was active in 3 sessions with objects recorded at the end of the experiment, after the sessions performed in the empty apparatus.
An observation made while analysing data was that all the place fields found in this study were located close to a wall or close to an object. An explanation for this might be that the walls and the objects are parts of the environment that have significance. The walls mark the boundaries and the objects are positioned inside the environment. Keeping track of where the borders are and what the environment contains is important.
Many studies have shown place fields located against the walls of the recording apparatus, e.g. Muller et al. [7], Breese et al. [33] and Hetherington et al. [62]. These observations suggest that a cognitive map made by the hippocampus does not consist of an even distribution of place fields in an environment.

## Intra-maze objects and place fields

Cell #1 in rat B2 and cell #2 in rat G2 both had place fields at the location of an object placed in the apparatus (Figure 18 and 16).
The cell located in rat G2 was lost before any further observations could be done. Cell #1 in B2 was stable, and responded to manipulations involving the object to which its firing field was attached. Firing fields attached to objects have also been reported in previous studies [46, 49]. Rivard et al. placed a barrier in an enclosure. When this barrier was rotated the firing fields of the cells located close to it moved accordingly. They named these cells object cells [46].
According to Rivard et al.'s study the closer the firing field is to the object it is attached to, the more likely it is that the field will disappear when the object is removed [46]. Our results do not correspond to their findings. Rat B2's object attached cell was attached to the can of paint, and when the can of paint was replaced the cell continued firing in the same place as previously, despite a new object being placed there. On the other hand, our results are in accordance with a study by Lenck-Santini et al. They showed that substituting an object with a novel one had no influence on place fields, located close to or far from the object [45].
Cell #2 found in rat G2 was also present in the apparatus containing objects (Figure 15). It had a place field which was located close to the apparatus wall. Unfortunately the cell activity in rat G2 disappeared before further observations could be done.

## Reward and place fields

In most studies of the properties of place cells reward has been given in order to maintain a high activity level, ensuring that the subject traverses as much of the test area as possible. Both rats B2 and G2 had stable place fields in the circular apparatus where reward was given (Figure 14 and 17). B2 also had a stable place field in the square apparatus containing reward (Figure 20).

When the rat was tested in this square apparatus with reward, after the sessions in the apparatus containing objects were recorded, a remapping occurred. Our data analysis has shown that thee cell present the apparatus containing objects was not the same as the cell present in the empty square apparatus. The difference in the spike waveforms can be seen in Figure 22. It is apparent that the waveforms changed between session 36 and 39 to such an extent that it is unlikely that the spikes originated from the same cell. It seems that cell #1 ceased being active, and in stead cell #2 appeared and developed a stable place field in the empty square apparatus.

From these observations we can conclude that stable place fields developed in both the reward and the no-reward version of the apparatus.

## Rotation of cue cards

Rotating local and distal cues is a common procedure to determine what factors influence the properties of a firing field. In our study we rotated the cue card to determine whether the place fields were based on the position of the cue card as a distal landmark.

For rat B2 sessions were recorded in the circular apparatus with the cue card relocated 180 °. Also sessions were recorded in the empty square apparatus with the cue card rotated 90° to the right.

The sessions recorded while the cue card was rotated 180 ° showed that the cue card was probably not significant for the placement of the place field (Figure 17). The place field remained in the same place when the cue card was moved, which means some other cue, most likely located outside the enclosure controlled that place field.

When the cue card was rotated 90 ° in the empty square apparatus the position of the firing field changed (Figure 20). However it did not shift according to the cue card rotation. The cue card was rotated 90° to the right; the field shifted its position 90° to the left, resulting in a new position in relation to the rotated cue card.

This implicates that the cue card was probably not the cue to which the firing field was tied If that was the case the firing field would shift according to the cue card rotation, neither is it consistent with the cue card being insignificant. If the rat only relied on extra maze cues the rotation of the cue card would not affect the position of the firing field (as seen in the cue card rotation in the circular apparatus). Still, as evident from Figure 20 the firing fields, both in the original scenario and when the cue card was rotated, are located in proximity to the cue card. However, since the testing enclosure is only 1x1 meter the continued proximity to the cue card might be incidental. Based on our observations it seems unlikely that the firing field is completely independent of or based solely on the cue card position. Most likely the position of the firing field is based on the cue card's position as well as the location of some other cues (most likely extra-maze cues). If that is the case this remapping corresponds to what is called partial remapping. In partial remapping only some cells respond to the induced change [36], making the new place field distribution dependent partially on the same set of cues as previously. In contrast a complete remapping is known by the firing fields' new positions being completely independent of their previous positions, and the firing fields can be controlled by a completely different set of cues. When we rotated the cue card 90° the firing field moved, but not according to the cue card rotation. It seemed to move from one location next to a salient cue to another location in proximity to the same cue. If this is the case, then the remapping is partial. However, if the continuing position of the firing field next to the cue card is incidental the remapping might be a global one.

### *Extra maze cues that might have been of significance*

The test apparatus was surrounded on all four sides by curtains. This should prevent the rat from seeing irrelevant cues. The curtain by the door sometimes remained open; which might lead to the rat being able to discern that wall from the other three. It is possible that the rat remembered which direction it came from seeing that it was allowed to look around when carried into the room and it was held for a while outside the apparatus while preparations for the session were made.

Other cues that definitely were available to the rats were auditory cues. The rats would have been able to hear the background noise made by the recording equipment and sounds made by the experimenters. The experimenters were always present next to the recording equipment and together with this they would probably act as a distal cue.

### *Theories and mechanisms that might explain our findings*

One would expect that cells found in sessions performed by the same rat reacted similarly to change. Particularly since the sessions were recorded in the same testing room and the access to extra maze cues should therefore be the same for the rat in both apparatuses. Dissimilar distance to the cue card could according to Rivard et al. [46] account for the different responses by the two cells. Their theory suggests that firing fields located close to the change would react more to it than more distant firing fields. We found that both firing fields were located close to the cue card, and their distance to the cue card is therefore most likely irrelevant.

In addition different theories on how stimuli control place cell activity have been proposed. The two main theories describing this is the rigid map scheme or the combinatorial scheme. In the rigid map scheme every cell is thought to be controlled by many types of stimuli which together make up the entire spatial reference frame. In the combinatorial scheme each cell is controlled by a subset of the stimuli received from the environment [47]. Assuming that the cue card is the only salient cue, according to both theories rotating the cue card would cause the cells' firing fields to rotate accordingly.

If the cue card is not the only salient cue, and something else is also important, the rigid map scheme entails that the firing field is based on a combination of the position of available cues, and complete remapping or partial remapping should occur. The combinatorial scheme entails that a remapping will occur if the field is controlled by the stimulus that is relocated (for instance the cue card), it will also remap if it is controlled by more than one stimulus and at least one of them is relocated and finally the field will stay in place if it is controlled by a stimulus which is not moved or altered.

When viewing our results in the light of these theories it becomes apparent that an explanation of us having dissimilar results from the same rat might be that the two firing fields were controlled by different cues. The cell present in the circular apparatus was most likely tied to extra maze cues, while the cell active in the square apparatus was probably controlled by a combination of the intra-maze cue card and extra-maze cues. When we relocated the cue card 90° the firing field did not simply follow the cue card rotation because the rotation put the salient stimuli in conflict with each other. Therefore it seems the relocation of the firing field was a partial remapping.

When the cue card was rotated back to its original location (see Figure 20) the cell's activity became lower and the firing field was less defined than previously. In addition the firing field

seems to have divided in two, one half moving back to approximately where the cell's firing field was located previous to the rotation, the other half staying where the place field was located when the cue card was rotated. This might also point in the direction of there being at least two salient cues, and that rotating the cue card put these cues in conflict with each other, making the field less distinct.

This kind of conflict between stimuli was explored by Tanila et al. In their study they recorded place cell activity in the CA1 and CA3 while moving separate distal cues in reference to each other. The cells recorded during this study showed a whole range of reactions to the cue manipulations. Some cells stopped firing, some cells' firing fields moved, some cells became active after cue manipulation and some cells' firing fields remained in place [63]. From these results they argue that different cells within a subset might be controlled by different cues and therefore respond differently to cue manipulations. Their interpretation of their findings is that the notion that the hippocampus forms a single cognitive map is not quite right, it seems like the map consists of submaps, each submap mapping the environment in accordance to one reference frame, or one set of cues. Studies performed by Gothard et al. and Zinyuk et al. supports this reasoning (See: *Spatial frames*) [42, 49].

## The firing rates of the isolated complex spike cells

To be able to draw any conclusions from the calculated overall firing rates one would need to know if the change in firing rate was significant. Due to the low number of sessions it is not possible to perform statistical analysis to assess the significance of any increase or decrease in overall firing rate.

The overall firing rate does not necessarily reflect any changes in the properties of the cell, changes in overall firing rate might be due to other factors.

The main problem with using overall firing rate in contrast to for instance in field firing rate is that the calculation of overall firing rate does not account for variations in the rat's activity level. If the rat spends its time outside the firing field most of the session the overall firing rate will be lower than if the rat spends a lot of time inside the cell's firing field. Any observations made on the variations in overall firing rate are therefore based on the assumption that the rat's activity in the testing area was evenly distributed and remained so throughout all the sessions.

Both Rivard et al. and Fenton et al. argue that when changes are introduced into an environment, causing a discrepancy between the expected and the actual appearance of the

surroundings, a rate remapping will occur. The rate remapping will consist of a decrease in firing rate [46, 47]. They also report that the firing rate will not be altered between standard sessions. In their studies the decrease in firing rate is mostly apparent in the in field firing rate and the firing rate at the field centre, the decrease in overall firing rate was a trend [46, 47]. They also argue that the in-field firing rate does not vary much between standard sessions.
In our study we found a steady decrease in overall firing rate for cell #1 for rat B2, from 1, 25 Hz in the initial standard session to 0,27 Hz in the last one (Table 2) . The decrease in firing rate was also apparent between the standard sessions. This finding is at variance with the previously mentioned findings of Rivard et al. and Fenton et al. [46, 47].
A major difference between the study by Fenton et al. [47] and our study was that their rats were trained in the same apparatus that was used in the experiment. The standard setup was used for the postoperative training, which consisted of 15-30 minute long sessions four to five times a day for a week. It can be speculated that this might lead to the rat being very familiar with its surroundings, and comparing all changes to this standard setup. In our study the rat was not introduced to the square apparatus containing objects before the recording period, and only two 5 minute long sessions were performed before changes were made. This might lead to our rat not considering the cues any more reliable in the standard sessions than in the others, and as more changes were made, the less salient the cues became and the firing rate abated.
For cell #2 in rat B2 there seemed to be an increase in the firing rate when the cue card was rotated, it seems that a rate remapping occurred. It most likely occurred in combination with a partial remapping (discussed earlier).

### Issues/ considerations

## Recording a high number of spikes

R3's clustering was difficult because of the large amount of spikes registered. As shown in Figure 23 the number of spikes within a cluster it was difficult to isolate spikes fired from a cell with a lower firing frequency than the spikes clustered here, particularly if the spikes had a similar waveform. This was unfortunate since spatially modulated cells fire with a lower frequency than the cells isolated here.

## Identification of place cell activity over a period of several days

A criterion for defining spikes recorded in two separate sessions as action potentials originating the same cell is that the cluster and the spike waveforms on the same electrode look similar. As Figure 22 shows the waveforms seen on the different electrodes within a tetrode can change between sessions. This might cause one to doubt whether or not the spikes recorded in two separate sessions originated from the same cell.

As can be seen in Figure 22 the waveforms from sessions 27 and 36 are not identical, with the signal on electrode 2 and 3 showing the most variation. The similarities are however also apparent, and when taking into account the signals recorded in the sessions between 27 and 36 the transition from the waveforms seen in session 27 to the ones seen in session 36 become apparent. This makes highly probable that the signals originate from the same cell. In addition the cell's firing field remained at the same location in the apparatus in all the standard sessions.

It is extremely difficult to keep the tetrodes completely stationary; they might be moved when the microdrive is being connected to the headstage, or when the rat bumps its head into for instance the cage wall. This could cause the signal registered on the tetrodes to change.

For rat B2 the first session and last session recorded in the apparatus containing objects, session 27 and session 46, respectively, were recorded eight days apart. This does not explain the discrepancy between the waveforms registered in this last session compared to the first one (see Figure 21). As the figure shows it is apparent that the waveforms of the spikes recorded on tetrode 3 in session 46 are similar to the previously recorded spikes in the empty apparatus originating, which originated from cell #2 (for instance session 39). This makes it likely that the spikes recorded in session 46 also originate from cell #2.

For rat G2 three sessions were recorded in the square apparatus containing objects before the cell activity disappeared. Assuming that tetrodes had shifted their position in the brain slightly we raised the tetrodes, trying to relocate the signal. Raising the tetrodes did not recover the signal, and neither did lowering them further.

The most likely reason for the signal disappearing is that the position of the tetrodes shifted. In that case the tetrodes would, in all likelihood, shift sideways, explaining why we did not relocate the signal by moving the tetrodes up or down. This assumption is supported by the fact that cell activity was located by lowering the signal further, meaning that there was nothing wrong with the connection between the tetrodes and the recording system.

## The use of reward

It is a known fact that stable place fields will develop in the presence of sufficient cues when the rat receives reward as a motivational factor. This was first shown by O'Keefe and Dostrovsky when they first recorded place cells in 1971 [6], and has been shown in numerous studies afterwards.
In this study our goal was to explore place field development and stability in the absence of food as a reward. We introduced objects into the testing apparatus to maintain high activity levels. The apparatus containing objects was defined as the no-reward condition. A possible problem with our testing procedure might be that the no-reward condition could have been influenced by the use of reward prior to and after the session. Reward was used to encourage the rat to sit still while preparing it for a recording session, and to disconnect the headstage from the microdrive at the end of a session. In addition reward was used in sessions during lowering of the tetrodes. This extensive use of reward might have led to the reward having a motivational effect in the sessions where we did not intend it to. Whether this had any effect on development of place fields is uncertain, the no-reward sessions were still without reward given directly.
It would most likely be possible to eliminate this memory of being given a reward after repeated sessions without reward, providing that no reward was used to prepare the rat for the session.

## Histology- Are the cells we registered the activity of CA1-neurons?

The brain was dissected out of the skull on both rat G2 and B2. The measured length of the tetrodes did not correspond to the calculated depth of the tetrodes for neither of them.
For rat B2 the calculated depth was 2.1375 mm and the measured depth was 2.9-3.0 mm. This was one of the largest discrepancies measured. The slides made from the brain of this rat shows the tetrodes' track ending in the CA1 of the hippocampus (see fig.26), which is located at an approximate depth of 2.2 mm [61]. This corresponds reasonably well with our calculated depth, especially when taking into account the inaccuracy present in the lowering of the tetrodes.
For rat G2 the brain was dissected out of the skull, but no histological preparations were made. This decision was based on the fact that the tetrodes were moved after the cells we recorded disappeared, and the position of the tetrodes when the search for complex spike

activity ceased were considered irrelevant. This was unfortunate; it would have been interesting to see where the tetrodes' track went, then we would at least have known where the recorded cells might have been.

From the histology results it is possible to conclude that the cells found in rat B2 were indeed spatially modulated complex spike cells located in the CA1-region of the hippocampus. The cells registered in rat G2 were recorded at a calculated depth of 1.95 mm, which according to the Rat brain atlas [61] is above the CA1, which is located at approximately 2.2 mm. There are however no known areas of the cortex above the dorsal CA1-region that contains place cells, and since the complex spike cells we recorded the activity of were the first complex spike cells we registered while lowering the tetrodes, they were most likely located in the CA1- area of the dorsal hippocampus.

For rat B2 and most likely rat G2 the spatially modulated cells we found were located in the CA1- area of the dorsal hippocampus.

## Test apparatus and procedures

When rat B2 was moved from the apparatus containing objects to the empty one a remapping occurred. Since the square testing apparatuses were identical in form the remapping could be due to the change in content. Another factor that possibly contributed to the remapping was the change in olfactory stimuli. The rat got to know one enclosure, for instance the one containing objects, and then this was replaced by the empty one, which lacked all the familiar smells.

Most likely the remapping was caused by a combination of several factors, mainly the change of apparatus and the change in content. The apparatuses were of the same shape, material and colour, but they were most likely still discernible for the rats. This was as we intended, the rat needed to recognise the enclosures as separate in order for us to conclude that stable place fields developed in both the reward and no-reward condition.

To further insure that the square apparatuses were similar but not identical the cue cards placed in them were of different colours. To make sure the cards did not influence the development of place fields differently they were all the same size and positioned towards the same side of the testing room.

### Interpretations of the results

Our main finding is that stable place fields developed in both rats that were tested in the reward and no-reward condition. Changes in position of the object to which the object cell was attached caused its firing fields to move accordingly. The exception being when the object was replaced, this did not cause any change in the firing field's position.
Changes in the orientation of the cue card had varying effects on the place fields' positions leading us to think that other cues than the cue card was available to the rat.
Unfortunately our results were obtained from only two rats and our results therefore limited. Our preliminary conclusion from our findings is that both the circular apparatus the square apparatus containing objects and the empty square apparatus have significant enough cues for stable place fields to develop and the conditions were different enough that the place cell population remapped when the rat was moved from one of the enclosures to the other.
The most interesting result is that stable place fields were found in the no-reward condition, which might indicate that the presence of food is not necessary for place fields to develop.
In our study two place cells developed place fields in the no-reward condition based on object position. The cell found in B2 showed hallmarks of being an object cell; when the object it was attached to was relocated the firing field shifted position accordingly. Object cells were described by Rivard et al.[46]. It is not known whether these cells are a subgroup of place cells that are always attached to objects present in the environment or if they are place cells that may be attached to an object in one environment and act as a "normal" place cell in another.
The object the place field was attached to was in no way connected to a reward. No reward was ever given in an environment containing that object, and the object had never been kept together with the reward or used to store the food pellets, which would have caused the object to smell like food. It therefore seems that the place field that developed did so based on the pure properties of the object.
When rotating the cue cards we found that two place cells in the same rat were probably controlled by different sets of cues, or spatial frames. And it seemed that for the cell controlled by more than one cue, putting those cues in conflict with each other caused a partial remapping and a rate remapping.

Finally; our calculated overall firing rates might indicate that the salience of the cues available in the apparatus containing objects for rat B2 decreased as manipulations were done to the environment. This was evident in the gradual decrease in overall firing rate.

## *Possible reasons for our problems locating complex spike cells*

In descriptions of studies done on place cells in the hippocampus very few if any mention the actual number of animals that was implanted, they usually only mentions the number of subjects included in the study. It is however likely that their success rate was higher than ours. There can be several reasons for not being able to find complex spike activity in an implanted rat. Some possible reasons will be discussed here.

### Unstable tetrodes

Towards the end of the study a small change in the construction of the microdrives were made which might have had some significance. The diameter of the inner cannula was changed. The new cannula had a slightly smaller diameter keeping the tetrodes closer together, which probably made them more immobile.
We suspect that the old cannulas probably allowed the tetrodes to move for instance when the rat bumped its head into the cage wall, which might have caused it to come in contact with surrounding cells, thereby damaging them. If the cells closest to the tetrodes were destroyed any signal picked up by the tetrodes would have to originate from cells located further away from them, causing the signal to be weaker. The new cannula probably kept the tetrodes more in place and might have prevented them causing tissue damage.
Both the rat G2 and R3 were implanted using microdrives containing this new cannula, rat B2 was not. Seeing that we got most of our results from rat B2 we can conclude that using the old cannula would not alone have been sufficient to prevent complex spike activity from being detected. It might also be that the change in microdrive construction was not critical at all.

### The resistance of the electrodes

Measuring of resistance is an important part of preparing microdrives for implantation.
It is done to evaluate the electrodes' ability to conduct electrical charges. High resistance causes low conductance and low resistance high conductance.

The optimal range of electrode resistance is between 200 kΩ and 500 kΩ. This can be difficult to achieve. Lowering of the resistance was done by platinising. It seemed to us that putting more than one layer of platinum on the tip of the electrode tended to make the resistance rise again. We used a current generator made in the lab to apply the platinum. Giving one pulse of

current did not cause the same drop in resistance in all electrodes. The result in the end was that some electrodes had a resistance above 500 kΩ and some below 200 kΩ. If the majority of the electrodes had a resistance within the preferred range the microdrive was implanted. Too high resistance in the electrodes might result in the signal recorded being too weak alternately too low resistance would cause background noise to be recorded. Both the resistance being too high or too low might cause cell activity to not be registered or to be overlooked while analysing the data.
Some of our implanted rats had electrodes with too much background noise and artefacts. Others had electrodes that seemed not to be in contact with cells, the signal being very low and often no (or almost none) artefacts were present when the rat groomed or chewed. If many electrodes registered too much noise or too little signal the chances of finding spikes originating from a complex spike cell were low. A lot of noise or almost no signal recorded corresponds with electrodes having too low or too high resistance, respectively. Still the electrodes showing signs of having too low or too high resistance during testing were not the same as the ones having the highest or lowest measured resistance prior to implantation. This might be due to the resistance changing during implantation or during the days the tetrodes were being lowered into the brain.

The larger area to conduct a current, the lower the resistance is. To minimise the resistance the tips of the tetrodes were carefully cut to give them the largest possible contact area. The tetrodes were also cut to the same length to make sure they reached the CA1- cell layer at the same time. Tetrodes with differing lengths might cause one tetrode to reach the cell layer before the others, causing us to stop the lowering of the tetrodes and preventing us from registering the surrounding cells that might have been recorded by the other tetrodes had they reached the cell layer simultaneously.

### Implantation

A reason for not finding any complex spike cells might have been that the tetrodes missed the target area. This does seem unlikely since the CA1-field of the dorsal hippocampus is a quite large area. When we calculated where to implant the tetrodes we aimed for the middle of the dorsal hippocampus, and there is ample room for a slight error due to imprecise measuring or variable size of the skull of the rats.

In a couple of rats we found, after dissection of the brain, that the tetrodes were slightly bent. In one rat they were bent to almost a right angle, barely entering the cortex. The reason for this error is not clear. The tetrodes are delicate, and it was easy to accidentally come in contact with them during the implantation, causing them to become bent. Slightly bent tetrodes might still reach the hippocampus, but it will make it difficult for us to calculate their real depth.

### Lowering the tetrodes into the brain

A problem we detected after having performed some histology was that the tetrodes were not lowered to the depth we had calculated them to be.

The lowering of the tetrodes after implantation was done gradually, and a depth protocol was made to keep track of the depth of the tetrodes position at any given time. The tetrodes were lowered slowly to minimise the damage done to the brain tissue and to reduce the risk of going past the CA1-area if our calculations should prove to be wrong. Notes were made of how much the screw on the microdrive was rotated, usually ¼ or $^1/_8$ of a circle, 50 or 25 µm, respectively.

A problem that became apparent after having performed some histology was that our calculated tetrode depth did not correspond to the measured length of the tetrodes protruding from the skull, which was measured after dissection of the brain. This might be caused by the cumulative error arising from the rotation of the microdrive screw. When turning it ¼ the error would be small, due to the fact that ¼ was the maximum distance one can turn the screw without needing to readjust the devise used to turn it. It is when the tetrodes are lowered $^1/_8$ (or in a very few cases $^1/_{16}$) of a circle that the problem arise. This is done by turning the screw halfway to ¼ of a rotation, and it relies purely on visual estimation, and this leaves room for an error. Rotating the screw $^1/_8$ of a circle gives only a slight error, but after multiple rotations $^1/_8$ of a circle, the accumulated error might become significant.

In addition driving screws on some of the microdrives are worn out, the spring that tensions the screw and prevents it from moving spontaneously, seems to become looser after the microdrive has been used several times. As a result of this it can be difficult to know if the wings of the screw are actually engaged and thereby leading the tetrodes into the brain or if the screw is just rotating without moving the tetrodes.

For the rats where the tetrodes were measured after dissection and discovered to be shorter than calculated this might explain the absence of multiple spike activity. This accounts for half of the rats. The other half of the rats had tetrodes that were measured to be longer than calculated, or approximately the length expected.

One possible explanation for the discrepancy that the measured length is slightly longer than the calculated length can be that the zero-point when calculating the depth of the tetrodes is the brain's outermost meninx, the dura mater. When measuring the length of the tetrodes protruding from the skull, the base of the skull is defined as zero. There is normally a slight gap between the skull and the surface of the dura mater, and this gap might cause the measured length to be slightly longer than the actual length of the tetrode that was inside the brain.

On the other hand, the post dissection measurement of the tetrodes protruding from the skull were also inaccurate, most likely more so than the discrepancy caused by the gap between the skull and the dura mater.

### Acute experiments

Due to our problems with recording complex spike activity we performed acute experiments to try to improve our success rate.

Both misplacement of tetrodes and unsatisfactory tetrode resistance should become apparent during acute experiments. We performed four acute experiments at different times throughout the study, and in all of them we found complex spike activity in the CA1-area. The same procedures for tetrode and microdrive preparation and implantation were used as for the animals that were part of the chronic experiments, the only exception being the fastening of the microdrives to the skull. This was not done in the acute experiment.

The pyramidal cells of the hippocampus have a relatively low activity level in awake animals. After anesthetizing a rat using urethane and lowering tetrodes into the CA1 the activity level was higher than would be seen in an awake animal. This is thought to be due to urethane's ability to inhibit the interneurons inhibiting the pyramidal cells.

The results from the acute experiments indicate that out implantation procedure was satisfactory and that the tetrode resistance we aimed to get was acceptable. The tetrodes implanted in these acute experiments were on one occasion used in a chronic implantation afterwards. This was the case for the tetrodes implanted on rat B2.

To sum up: The absence of complex spike activity can be explained by the tetrodes missing the hippocampus, not reaching the CA1-area, the resistance being too high or to low or the tetrodes destroying the cells surrounding them because of their movement.
It is difficult to explain why we found complex spike activity in so few of our subjects. The fact that our implantation protocol remained unchanged during the study and that acute experiments performed yielded such good results makes it difficult to pinpoint exactly what the cause of our problems finding complex spike activity might have been

## *Future perspectives*

Our main finding was that stable place fields seemed to develop both in the presence and absence of reward. In addition the cells seemed to react to manipulations of objects in a manner that is characteristic of postulated place cells.
More results are needed to confirm our findings. Future studies should eliminate some of the sources of error present in this study, for instance the fact that we used reward in the circular apparatus while searching for spatially modulated cell activity, and that the rat was given food to sit still while preparing it for the session. This could possibly be achieved by restrict use of food in the experimental setting, thereby preventing any confusion between the reward and no-reward condition.
It would be interesting to see if introducing food into the testing apparatus containing objects would have any effect on established place fields, alternatively introducing objects to an empty testing apparatus where food has been used to establish stable place fields to see whether that would cause a change in the place cell activity.
Likewise it would be interesting to study place field development in an empty apparatus where no reward or objects are present. This might give some insight into the place field dynamics in the absence of any apparent motivational factor. In such experimental conditions there is of course the problem of making sure the rat traverses the area sufficiently.

The success rate of the implantations should improve before any more studies using chronic implanted tetrodes are initiated.

There is a lot of interest in how the brain processes the information it receives about the outside world via the senses, and how it gathers this information into a representation of the world surrounding it. The hippocampus seems to have a key function in this process.
The functions of the hippocampus in humans can be studied through functional studies, like PET or fMRI. The functions of the different cell types, their connectivity and their reaction to different stimuli can however not be studied to any great length in humans.
Seeing that the human and rat hippocampus is quite similar one can study these factors in the rat brain and thereby get an insight into how the human hippocampus works at a cellular level.
Studies on place cell remapping may also be important because they show how the brain can make separate representations of similar environments, a mechanism which also might be used to separate non-spatial memories.

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# Appendix

## Experimental subjects

**Table 6. The arrival date for the subjects, surgery date, testing period, recording period and date of euthanasia (and perfusion if this was performed)**. The recording period is only listed for the rats in which we found place cell activity.

| *Rat* | *Arrival date* | *Surgery date* | *Testing period* | *Recording of place cells* | *Euthanasia (perfusion)* |
|---|---|---|---|---|---|
| ***R1*** | 09.07.08 | 08.01.09 | 12.01.09-03.03.09 | | 04.03.09 |
| ***R2*** | 09.07.08 | 13.01.09 | 15.01.09-18.03.09 | | 20.03.09 |
| ***R3*** | 09.07.08 | 19.02.09 | 23.02-09-02.04.09 | | 08.05.09 |
| ***R4*** | 09.07.08 | 07.01.09 | 12.01.09-03.03.09 | | 04.03.09 |
| ***B1*** | 09.07.08 | 09.10.08 | 16.10.08-28.11.08 | | 01.12.08 |
| ***B2*** | 09.07.08 | 18.02.09 | 19.02.09-03.04.09 | 07.03.09-03.04.09 | 03.04.09 |
| ***B3*** | 09.07.08 | 14.01.09 | 20.01.09-11.02.09 | | 11.02.09 |
| ***B4*** | 09.07.08 | 04.11.08 | 12.11.08-15.12.08 | | 15.12.08 |
| ***G1*** | 09.07.08 | 11.02.09 | Acute experiment | | |
| ***G2*** | 09.07.08 | 25.02.09 | 27.02.09-30.03.09 | 05.03.09-06.03.09 | 30.03.09 |
| ***G3*** | 09.07.08 | 29.09.08 | 03.10.08-04.12.08 | | 04.12.08 |
| ***G4*** | 09.07.08 | 14.10.08 | 24.10.08-11.12.08 | | 11.12.08 |
| ***S1*** | 09.07.08 | 23.09.08 | 30.09.08-04.12.08 | | 04.12.08 |
| ***S2*** | 09.07.08 | 10.11.08 | 13.11.08-30.01.09 | | 30.01.09 |
| ***S3*** | 09.07.08 | 02.02.09 | 05.02.09-27.03.09 | | 30.03.09 |
| ***S4*** | 09.07.08 | 28.01.09 | 02.02.09-20.02.09 | | 20.02.09 |