Behavioural and Physiological Responses of Rats to Acoustic Boundaries

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I affirm that I have written the dissertation myself and have not used any sources and aids other than those indicated.

Date/Signature: _____

Contents

Abstract

In recent studies on rodent spatial cognition a special role was ascribed to boundaries in the environment (O'Keefe and Burgess, 1996; Hartley et al., 2000; Barry et al., 2006). They were proposed to determine the position and shape of place fields. It was even suggested that the emergence of place fields requires the presence of stable boundaries (Jeffery, 2007). However, it is not clear what stimulus constitutes a boundary. A recent experiment showed that boundaries do not need to be implemented as physical walls. Hayman et al. showed that rats can be efficiently constrained to an experimenter-defined area ("silent zone") by playing loud noise whenever the animals crosses an invisible boundary ("noise boundary") (Hayman et al., 2008). Thus, noise boundaries seem to be a promising tool to impede movement in environments lacking physical walls, such as virtual environments. The aim of this study was to characterize the effects of noise boundaries on behaviour and on hippocampal place cell responses prior to their application in a rodent virtual reality setup.

I found that noise boundaries did not constrain the animals' range perfectly to the silent zone. However, the rats showed a strong preference for it, undertaking short strays to the remaining parts of the arena only occasionally. The more experienced the rats became in the setup the longer and the more frequent these strays became, i.e. the animals adapted to the noise. Furthermore, I could show that the animals were capable of finding the silent zone in the arena even when it was not cued by any landmark (place learning). In accordance with previous studies on place learning (Morris, 1981) this finding was interpreted as an indication that the silent zone was represented in an internal model of the physical environment ("cognitive map").

I aimed to clarify whether the cognitive map included the exact geometry of the silent zone or just the approximate location. I could not find indications for the hypothesis that the rats had learned the exact spatial layout of the zones. They did not show a tendency to move along the noise boundaries inside the silent zone, as it is the case with physical walls. Moreover, I could not find place cells encoding the rat's position relative to the noise boundary. Therefore, I suggest that noise boundaries contribute to spatial cognition in a different way than physical boundaries, as they probably do not allow for the formation of metric models of the spatial environment.

1   Introduction

1.1   The Cognitive Map

In 1948 Edward Tolman provided evidence that rats are able to take shortcuts and roundabout routes when navigating mazes (Tolman, 1948). His discovery lay the grounds for the cognitive map theory, stating that animals posses mental representations of their spatial environment which help them to efficiently solve spatial problems. This idea contrasted sharply with the then prevailing behaviourist approach, which sought to explain behaviour as a pure stimulus-response linking. Despite the opposition the cognitive map theory faced when it was first proposed, it is now one of the most recognized concepts in spatial research. The main reason why it gained such popularity was the discovery of a neuronal structure, the major function of which seems to be the representation of space: the hippocampal place cell system. Hippocampal place cells are principal cells of the CA1 and CA3 regions of the rat and mouse hippocampus (O'Keefe and Dostrovsky, 1971). These cells increase their firing rate dramatically whenever the animal is at a specific location (the place field), independent of heading. Place cells were claimed to be the neuronal building-blocks of the system underlying the cognitive map.

O'Keefe and Nadel envisioned the cognitive map to be much like a geographic one: a complete allocentric representation of space which faithfully depicts distances and angles between objects (O'Keefe and Nadel, 1978). Furthermore, they claimed that the representation of space is the major function of the pyramidal cells in CA1 and CA3. Both of these ideas have been much criticized and are still under debate (for reviews of alternative viewpoints see Eichenbaum et al. 1999; Redish 2001). Although there is currently no agreement on how exactly spatial relationships are represented in the rodent brain, the existence of such representations is generally believed in.

1.2   Internal Models of Space and Rodent Navigation

Complementary to the discoveries made in neurophysiology there are observations on the behavioural level which support the idea of an internal representation of space. Besides the ability to shortcut (Tolman, 1948) the most convincing argument in favour of such representations is the fact that rodents can learn to navigate to or away from places which are not directly marked by any cue. In these situations the location of the target needs to be inferred from the position of distal cues. Alternatively, the animal may keep track of its own position relative to the target by integrating all translations and rotations it underwent since it last encountered the target or a reference point with a known spatial relationship to the target (Golledge, 1999). This process is known as path integration. No matter whether distal landmarks or path integration is employed, successful navigation to an unmarked target always implies some form of knowledge regarding the spatial relationship between the target and distal landmarks or the target and oneself, respec-

tively. Arguably, such knowledge may be far away from a holistic and metric representation of space.

When animals learn to navigate to or to avoid unmarked places one speaks of place learning, as opposed to cue or beacon learning, in which the animal simply learns to move towards a landmark in order to reach a target (Golledge, 1999). Place learning occurs for example in the Morris water maze (Morris, 1981). In this paradigm rats have to find a platform in a pool filled with opaque liquid which can only be perceived at close distance. After some training rats swim straight towards the platform from any start position in the water. The fact that performance in the Morris water maze is heavily impaired in rats with total hippocampal lesions has been taken as evidence that the cognitive map is encoded by the hippocampus (Morris et al., 1982).

1.3   Determinants of Place Fields

In the past three decades many studies have tried to determine what information is used to create internal models of space and how different sources of information interact in this process. What exactly causes a place cell to fire when it fires?

Place cells have been shown to be sensitive to wide range of environmental manipulations such as changing the colour of a cue card (Bostock et al., 1991), the shape of the box (Lever et al., 2002), the colour and odour of the box (Anderson and Jeffery, 2003) or placing the recording box in a new place (Hayman et al., 2003) or room (Leutgeb et al., 2005). Some manipulations alter the shape and position of a subset of place fields ("partial remapping") while other manipulations lead to a completely unrelated distribution of place fields across the environment ("complete remapping") (Kubie and Muller, 1991).

Studies on remapping have revealed that on many occasions place fields follow salient cues when they are moved and disappear when these cues are removed (e.g. Shapiro et al. 1997), suggesting that the presence of a conjunction of cues might trigger discharge in an absolute fashion. However, the idea of sensory control has been disproved by studies revealing that experience exerts a strong influence on place fields (Quirk et al., 1990; Sharp et al., 1990; Markus et al., 1994). For example, place fields tend to follow a salient visual cue that is moved in between trials but cease following it when the cue is experienced to be mobile (Jeffery, 1998). Thus, it seems that the critical features which trigger place cell firing are selected dynamically from numerous sources of information, depending on experience (Jeffery, 2007).

In addition to experience there are also other contextual factors which are known to influence place fields. Markus et al. reported that pace fields may remap when the rat switches to a different task, even though the environment remains unchanged (Markus et al., 1995).

More recent studies suggest that place cell activity correlates with higher cognitive processes such as planning, recall and shifting attention. These studies mostly relate to the activity of place cells observed when the animal is currently outside the place field (for a review see Johnson et al.

2009). There is, for example, mounting evidence that sequences of activations in hippocampal place cell ensembles which occurred during spatial behaviour are repeated during certain sleep phases (e.g. Louie and Wilson 2001; Skaggs and McNaughton 1996; O'Neill et al. 2008). Similar sequences of activations have been observed when rats pause before choosing a route at an intersection in the maze (Johnson and Redish, 2007). In these cases the sequences do not code for routes the animal has already taken, as in replay, but seem to represent potential future routes. Recently it was reported that some cells that have place fields during exploration encode route choices during delay periods (Pastalkova et al., 2008). As the rats were held back at an intersection in the maze for a short period of time some cells only fired if the rat took a certain route afterwards.

1.4   The Role of Geometry

Owing to the ever growing list of factors which may influence place cell activity the modelling of place cells is a challenging task. As a first step it seems reasonable to look for the determinants of place fields in the simplest of circumstances, i.e. in plain environments without any task and previous training. It was found that in such a situation the geometry of the environment is a critical factor. Burgess and O'Keefe recorded place cells in four rectangular boxes that differed only in the length of one or both sides (O'Keefe and Burgess, 1996). Interestingly, they found systematic changes in place field position and shape when the rats were transferred from one box into another. Most of the observed place fields kept either a fixed distance or a fixed proportion of distances to a box wall in the directions defined by the axes of the box. In many cases place fields that were circular in the small, square box were elongated or split in two in the bigger boxes. Figure 1 shows examples of how fields differed in the different environments. Burgess and O'Keefe explained the changes in place field shape and position by assuming that place cells receive input from cells that are sensitive to the distance of the rat from boundaries in the environment (O'Keefe and Burgess, 1996). They termed these putative cells "boundary vector cells" (BVCs). These cells were proposed to discharge maximally whenever a boundary is at a particular distance and allocentric direction from the animal. For example, a BVC might discharge maximally whenever there is a wall at 20cm east from the animal (see figure 2). The response properties of these cells were proposed to be independent of heading. The activity of a place cell was claimed to be the thresholded sum of all the BVC inputs that particular place cell receives. Hartley et al. examined the responses of place cells in one environment and chose the combination of BVC inputs that best explained the responses of each place cell (Hartley et al., 2000). By computing the thresholded sum of the responses of the chosen BVCs to a novel environment they were able to predict basic responses of the place cells to that novel environment, such as place field shift, elongation or splitting.

The boundary vector cell model received much attention recently when Solstad et al. reported
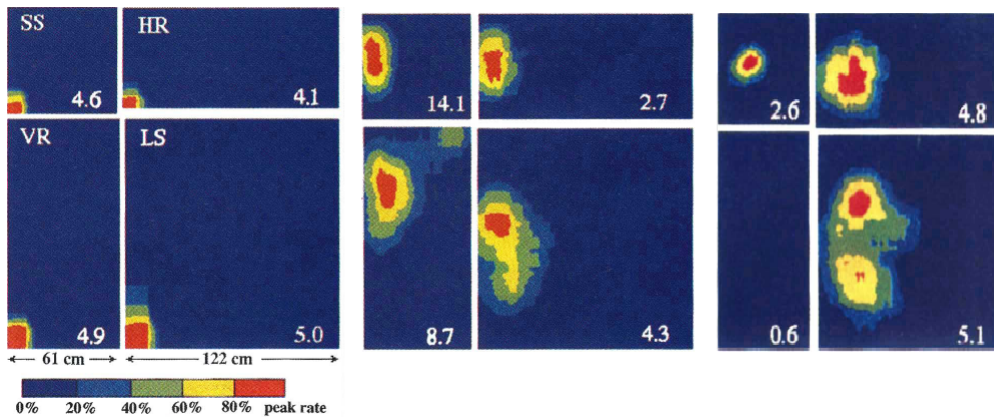
Figure 1: **Effects of changing the geometry of the environment on place fields. Firing rate is displayed in colour-coded contour plots (rate maps). The white numbers indicate peak firing rates. Three place cells were recorded in four different rectangular environments (SS: small square, HR: horizontal rectangle, VR: vertical rectangle, LS: large square). The field to the left occurs at a fixed distance from the west and from the south wall. The field in the center occurs at a fixed distance from the west wall and a fixed ratio of the distance between the north and south wall. The field to the right divides as the rat is transferred to the large square. From O'Keefe and Burgess 1996.**
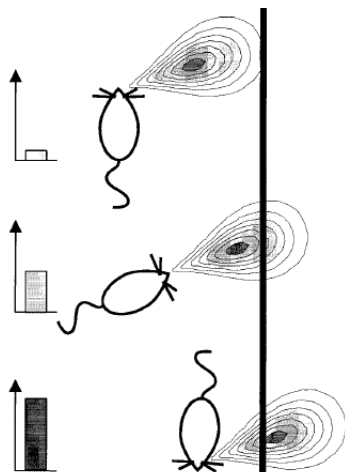

Figure 2: **The activity of the putative boundary vector cells (bar charts on the left) is maximal when there is a boundary at their preferred distance and allocentric bearing, independent of heading. From Hartley et al. 2000.**

the discovery of cells in the entorhinal cortex which have the properties Burgess and O'Keefe ascribed to their putative BVCs (Solstad et al., 2008). These cells fire exclusively along one or several boundaries of the enclosure, no matter whether the boundary is a wall or a drop at the edge of platform. They discharge at a fixed distance to all walls at a specific allocentric direction and keep doing so when the shape of the enclosure is changed.

1.5   The Fuzzy Boundary Arena

Given the importance of boundaries for the responses of place cells the question arises what stimulus constitutes a boundary in the first place. Obviously boundaries are characterized by impeding movement in a particular direction. The boundaries a laboratory rat encounters in an experimental setup are usually the walls of arenas or mazes or drops at the edges of platforms. However, boundaries may be implemented quite differently, as shown in a recent study by Hayman et al. (Hayman et al., 2008). They tracked rats while they explored a large area surrounded by curtains in near darkness. Whenever the animals moved too far away from the centre of the room and crossed an invisible, experimenter-defined boundary aversive white noise was played (80dB). If the rat continued to walk towards the periphery and crossed a second invisible boundary the sound pressure was increased to 95-100dB (the area beyond the second boundary is henceforth referred to as the "noisy zone"). This way the animal's exploratory range could be constrained effectively to the central part of the room ("silent zone"). Importantly, the position of the inner boundary was reset frequently within a certain zone ("adjustment zone") so that the rat could not predict the onset of the noise as it moved outwards.

With regard to the constantly changing position of the inner boundary Hayman et al. termed their setup the "fuzzy boundary arena". Figure 3 shows a scheme of the setup and the normalized occupancies (time spent in zone/area of the zone) for the silent, the adjustment and the noisy zone.

In addition to the behavioural variables Hayman et al. also assessed neurophysiological responses in the fuzzy boundary arena. They investigated place cell activity in five different conditions: In the first condition the rats were kept inside a small box that was located in the silent zone for the entire trial. In the other four conditions parts of the box (pillars and walls) were removed at the beginning of the trial so that the animal could leave the box and explore the arena (see figure 3). It was observed that as the walls of the box were removed one by one the place fields disintegrated. The more box walls were missing the less localized spatial firing became.

The effect of wall removals on place fields in the fuzzy boundary arena is described in more detail in another article published by the same group (Barry et al., 2006). There it is stated that out of 25 place cells which had well localized fields in the box only two cells had fields when only one pillar of the original box was present. These fields followed the pillar when it was
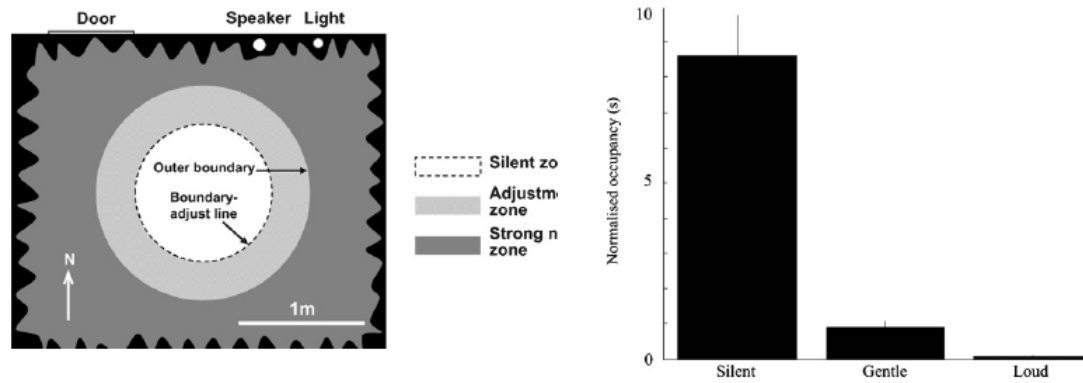
Figure 3: **Left: Experimental room showing initial location of sound barriers. The outer, dark grey area shows the strong white noise zone and the inner, light grey circle shows the gentle white noise zone. Wavy lines at the edge depict black floor-to-ceiling curtains. Line at bottom right of figure shows scale of room. Right: Normalized occupancy (time spent in zone/area of the zone) for each zone with standard error bars. From Hayman et al. 2008.**

moved between the trials. Therefore it was suggested that localized place cell activity might require stable boundaries (Jeffery, 2007). When physical walls are not present and the stimulus that impedes movement, namely the noise boundary, is moving constantly, the boundary cells might produce incoherent output, resulting in place field disintegration.
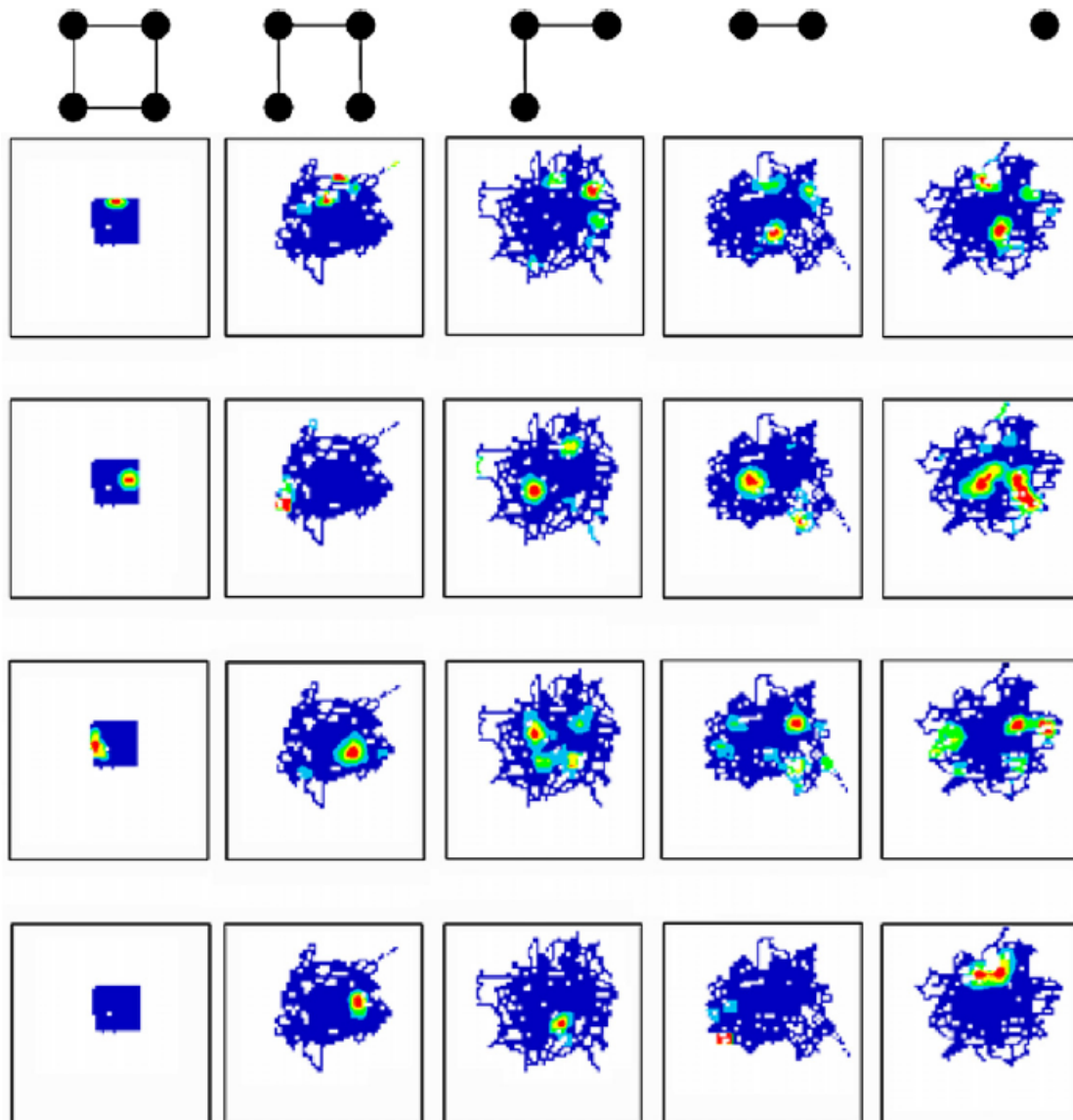
Figure 4: **Effect of wall removal on four place cells. The activity of the cells is shown as a contour plot (high activity = hot colors, low activity = cold colors). Manipulation to the local environment is shown at the top (four walls, three walls remaining, etc.). Note that the activity of the cells is tightly localized when all four walls are present, and tends to be close to the walls. As walls are removed the firing becomes less well localized. From Hayman et al. 2008.**

2   Experiment 1 - Testing the Fuzzy Boundary Arena

2.1   Motivation

The fuzzy boundary arena was described as an effective tool for restraining a rat's range in an environment without physical boundaries. Therefore it might be useful for place cell recordings in virtual worlds which lack physical walls by nature. Our group has presented such a setup and showed that rodents can successfully navigate in virtual environments (Hölscher et al., 2005). In this setup the rat is held on top of a polystyrene sphere floating on an stream of compressed air (Figure 5). The sphere is surrounded by a toroidal screen. Any translational movement of the animal leads to a rotation of the sphere, which is monitored and fed to the computer that controls the generation of the virtual environment. The virtual world is rendered and presented to the animal on the screen in a closed action-perception loop. The setup enables us to investigate navigation in environments of arbitrary size. For place cell recordings, however, it is necessary that the rat is constrained in its range so that it visits all places repeatedly, otherwise unit activity cannot be correlated to the rat's position.

Applying noise boundaries might also help to make virtual environments more immersive which would otherwise consist exclusively of visual stimuli. As rats are known to rely much less on vision than humans, additional features of a different modality are likely to aid them in navigating the virtual environments.
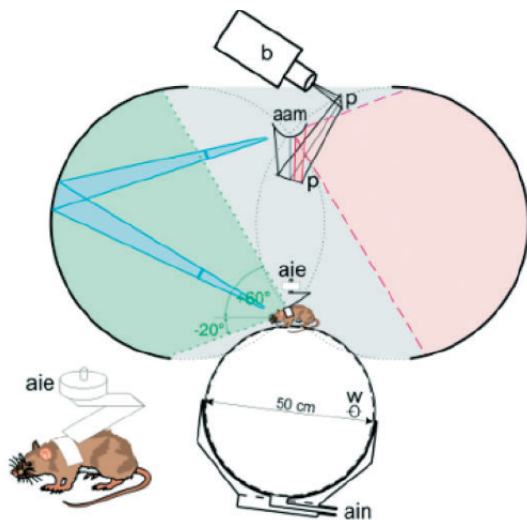
Figure 5: **Scheme of the rodent virtual reality setup developed by our group. Abbreviations: aam = angular amplification mirror, aie = angular incremental encoder, ain = air inlet, b = projector, p = plane mirror, w = wheel. From Hölscher et al. 2005.**

The motivation for the first part of this study was to assess the possibility to transfer the methods of the fuzzy boundary arena to our virtual reality setup. The first aim of this study was to reproduce the results of Hayman at al., i.e. to show that it is indeed possible to constrain a rat's

range by using noise boundaries. I was especially interested to see whether the noise remained effective when applied repeatedly.

2.2 Materials and Methods

2.2.1 Subjects

Two adult male Long–Evans rats *Rattus norvegicus* (Charles River, Germany), weighing 130-150g at the beginning of the experiments served as subjects (rats 154 and 156). The animals were naive to the paradigm and neither food- nor water-deprived. All experiments were licensed according to German and EU law regulating the use of animals in research.

2.2.2 Experimental Room

The experimental room was 3m x 4.1m in size. In the center of the room was an 1.8m x 1.8m wooden arena with black walls, surrounded by black curtains (see figure 6). The ceiling was also covered with black cloth, forming a closed compartment with the curtains to the side. The floor was covered with black polypropylene sheets which were wiped with ethanol regularly to minimize the influence of odour cues. Centered above the arena on the ceiling there was a standard black and white camera for tracking and a 5W light bulb for low-intensity illumination. Above the cloth ceiling there was a rack attached to ceiling of the room holding the audio-amplifier and the pre-amplifier for spike recording. The cable used for recording reached down through a hole in the ceiling cloth. The two custom loudspeakers used to generate the white noise were located in a rack just next to one corner of the arena, at a height of approximately 1.5m. The computer used for tracking and spike recording was in the same room as the arena.

2.2.3 Local Environment

The arena was divided into three zones as in Hayman et al. 2008. In the silent zone no noise was ever played. The silent zone was a square of 76cm x 76cm in the center of the arena (see figure 6). It was marked by a metal pole of about 20cm in height painted with fluorescent colour to improve its visibility in near-darkness. The landmark always appeared at the same position within the silent zone (approximately in the center). The boundaries of the silent zone, as well as all the other sound boundaries, were defined in tracker coordinates and were not cued in any way. The adjustment zone (area: $0.8m^2$) formed a square frame around the silent zone with a width of 20cm. It was itself surrounded by the noisy zone (area: $1.92m^2$). The inner boundary changed its position every 2s within the adjustment zone and is therefore termed the fuzzy boundary. When the animals crossed the fuzzy boundary white noise with a sound pressure of 84dB was played. Thus, one part of the adjustment zone was usually silent while entering the remaining part led to the playback of noise. Crossing of the outer boundary (between adjustment

and noisy zone) triggered the playback of even louder white noise (94dB). The outer boundary did not change its position.
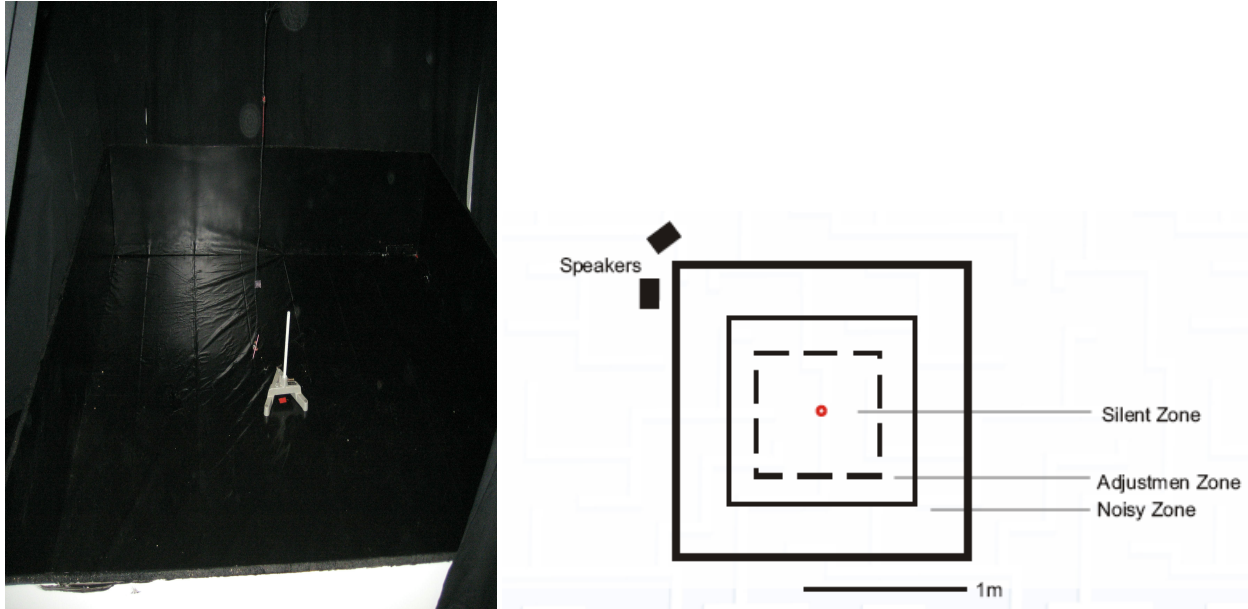

Figure 6: **Left: Photograph of the arena used in the experiments. The wooden arena was surrounded by black curtains and lined with black plastic sheets. The landmark cued the silent zone. The cable for spike recordings reached down from the ceiling (not used in experiment 1). Right: Scheme of the arena. The fuzzy boundary (dotted line) changed its position within the adjustment zone frequently. In this picture it is located at the innermost position which could be taken. The outer noise boundary (thin black line) separated the adjustment and the noisy zone. It always remained at the same position. The red circle represents the landmark.**

2.2.4 Tracking

The rats wore custom-made leather jackets bearing hook-and-loop fasteners on their outsides (see figure 7), facilitating the attachment of an infrared LED with a small battery. The LED was tracked by the camera on the ceiling.

Tracking was facilitated by the hard- and software system Dacq2 (Axona Ltd., St.Albans, UK).

2.2.5 Procedure

Two rats were tracked in my version of the fuzzy boundary arena in 16 trials each. There were two trials per work day. In addition, eight trials (four per rat) were run in the arena without playing any noise. These trials served as a control.

Before the start of each trial oat flakes were distributed in all parts of the arena to encourage movement. Next, the rat was put into a small, wooden transport box which was then placed
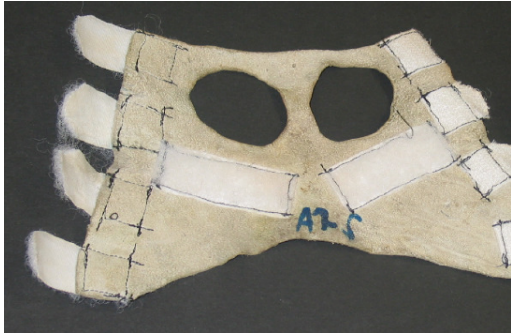
Figure 7: **Photograph of a leather jacket custom-made for experiments with rats.**

in the center of the silent zone. The lights were switched off, except for the 5W bulb, the trial was started and the rat was placed just next to the transport box. Then the transport box was removed. A trial lasted 10 minutes. At the end of each trial the light was switched on, the rat was picked up from wherever it happened to be at that point and was put back into the transport box.

2.2.6   Analysis

For the comparison of data from two different samples the standard two-sample t-test was used in case the Lilliefors test did not call for a rejection of the hypothesis that the data in the samples were normally distributed. Otherwise the Wilcoxon rank sum test was used. Given in the results sections are the resulting p-values (p).

To test for effects of factors standard regression analysis was applied. The abbreviations appearing in the results section indicate the slope of the regression line fitted to the sample (r), the confidence interval for the slope of the true regression line (CI) and the probability that the true slope is equal to zero (p).

All analyses were performed with Matlab (The MathWorks). Extracting the position data from the Dacq position files was achieved with the help of a Matlab script from Sturla Molden, Centre for the Biology of Memory, Norwegian University of Science and Technology. The procedure involved the application of a Kalman filter. The filter interpolated the data and removed implausible outliers. Following the application of the filter one obtained about 50 data points per second.

2.3   Results

2.3.1   Raw Data

The effectiveness of the noise boundaries is evident from looking at the raw data (see figure 8). The rats mostly stayed inside the silent zone when the noise boundaries were applied and visited the other zones only occasionally. While the rats did not reach the walls of the arena in some

trials they made longer strays to the noisy zone in other trials, including periods of movement along the physical walls. In the control trials movements along the walls of the arena dominated the paths. In both, test and control trials, there were periods in which the rats were occupied with the landmark.
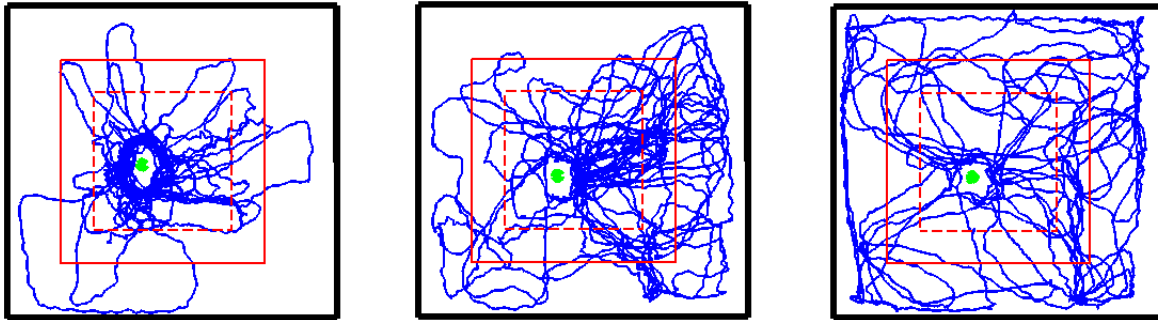

Figure 8: **Three selected trials. The path of the rat is shown in blue, the arena walls in black, the landmark in green and the noise boundaries in red (dotted line marks the innermost position the fuzzy boundary may be at). Left: The path of subject 2 on its first trial in the fuzzy boundary arena. The animal stayed mostly in the silent zone but made occasional visits to the other zones. Middle: The ninth trial of the same animal. Visits to the noisy zone were more frequent. Right: A control trial of the same animal.**

2.3.2   Occupancy and Activity

Prior to presenting the occupancies (dwell times) for each zone I shall comment on the form of their presentation. I did not assess the effect of the aversive noise in the same was as Hayman et al.. They compared the normalized occupancies (dwell time in zone/area of the zone) in the three zones (Hayman et al. 2008; compare section 1.5). Because these were not equal in all of the zones they concluded that the noise was indeed effective. The rationale being that without noise the zones would be visited according to their area. Analysis of behaviour in the control condition revealed that this assumption is not valid.

In the control trials the rats spent on average half of the time in the noisy zone and a quarter of the time in the silent and the adjustment zone each, even though the adjustment zone covered a greater area than the silent zone (24% of the total area versus 18%; see figure 9). A one sample t-test rejected the hypothesis that the true mean of the occupancy in the silent zone is equal to 18% of the total occupancy (p=0.02). A similar result was obtained for the mean occupancy in the noisy zone (p=0.008) where the rat spent less time than predicted by the area. These observations show that it is not appropriate to normalize the occupancy in a zone by dividing by its area. Instead, one needs to compare the occupancies in the presence of noise to the occupancies in the control condition in order to assess the effect of the noise boundaries.
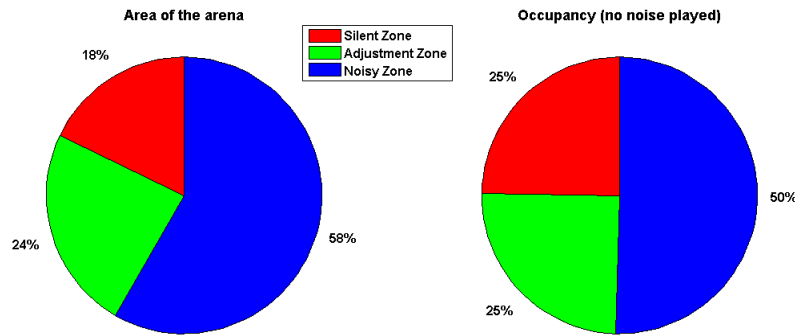
Figure 9: **The pie chart to the left depicts how the area of the arena is distributed across the zones. The chart on the right shows the distribution of occupancy across the zones in the control condition. Despite the similarity, the distribution of occupancy differs significantly from the distribution of area.**

Thus, I calculated the average occupancy for each zone and compared it to the corresponding value from the control trials. The result is shown in figure 10. Both animals spent most of the trial time inside the silent zone only when the noise was present.

In addition to occupancy I assessed the activity of the subjects in each zone. In order to measure activity I divided the arena into 100 square bins of equal size (18cm x 18cm) and counted the number of bins the rat passed per unit of time. Back and forth crossings between two bins were ignored, as they may result from small movements made for example during cleaning behaviour. Activity in the silent zone was significantly reduced when noise boundaries were applied.

2.3.3   Adaptation

I was curious to see whether there were indications for an adaptation to the noise. Visual inspection of the paths suggested that visits to the noisy zone were more frequent in later than in earlier trials (compare figure 8, middle picture). Indeed, one of the two animals spent more time in the noisy zone in later trials than at the beginning of the experiments (see figure 11). The effect was confirmed by standard regression analysis ($r = 11.8, CI = [7, 16.4], p = 0.0001$). For the activity (average across zones) I did not find a significant effect. The other animal showed adaptation up to the tenth trial but then reduced its activity strongly and rested mostly in the silent zone, resulting in a significant effect of trial number on activity ($r = -0.06, CI = [-0.09, -0.03], p = 0.001$) but not on occupancy ($r = 3.1, CI = [-22, 101.1], p = 0.32$).
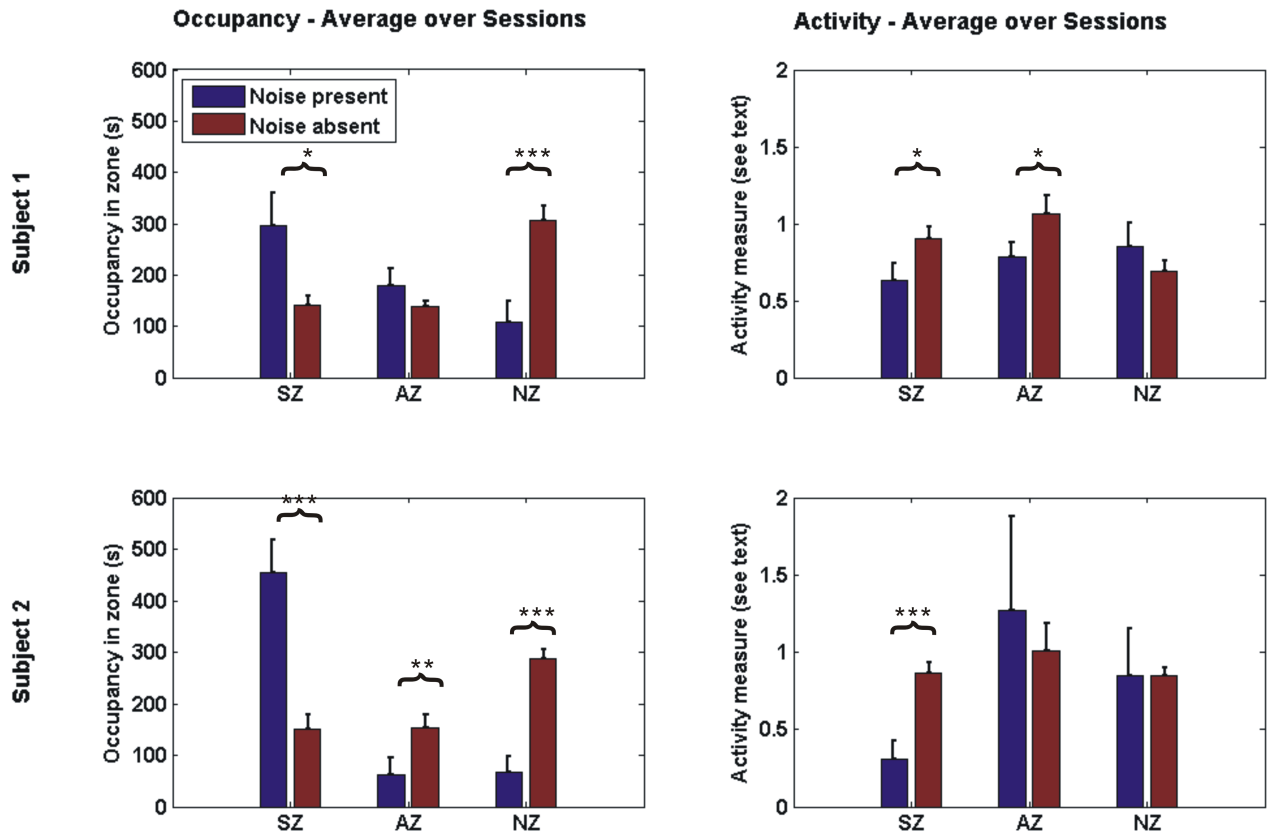
Figure 10: **Average occupancy and average activity in the silent zone (SZ), the adjustment zone (AZ) and the noisy zone (NZ). The errorbars indicate the standard error of the mean (SEM). Values obtained when the noise boundaries were present are depicted in blue, values from the control condition are displayed in red. P-values are symbolized by stars:** $* \implies p < 0.05, ** \implies p < 0.01, * * * \implies p < 0.001$.

Figure 11: **The development of occupancy in the noisy zone and the average activity over trials. There was a significant positive effect of trial number on occupancy for subject 1 and a significant negative effect of trial number on activity for subject 2.**

2.4   Discussion

The results show that noise boundaries are indeed an effective tool for constraining a rat's range in environments without physical bou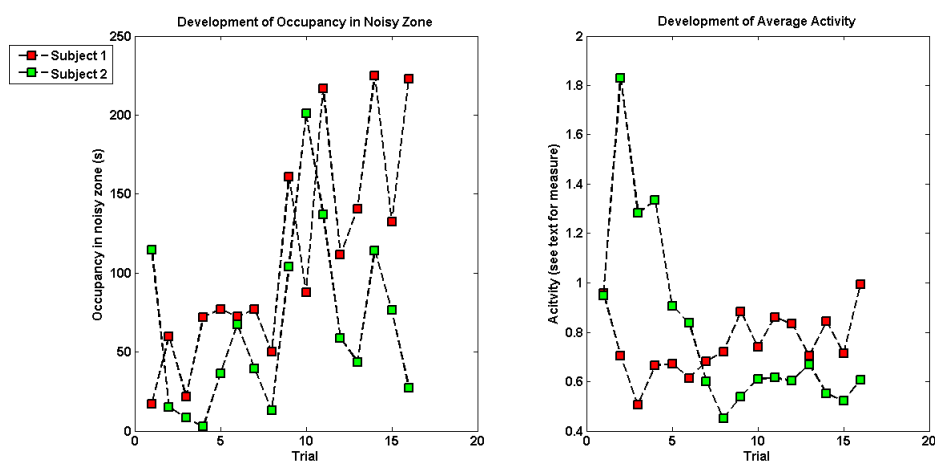ndaries. Therefore, they qualify as a potential tool for neurophysiological and navigational experiments in virtual environments. However, noise boundaries are not perfect replacements for physical boundaries. They do not prevent the animals from exploring a certain compartment of the arena entirely. In all trials the rats made excursions from the silent zone to the noisy zone. Although there were trials in which the animals did not reach the walls of the arena – especially when the rats were unexperienced in the setup – the noise boundaries did not prevent them from doing so in general. In conclusion, the noise boundaries did not act like physical boundaries but rather led to a strong preference for the silent zone.

In contrast, Hayman et al. reported that "the barrier was very effective in preventing the rats from coming into contact with the experimental room walls" (Hayman et al., 2008). They may have come to this conclusion because they used a much bigger arena than I had. In my setup the rats could probably see the arena walls from all positions in the arena, which may have been different in Hayman's setup. Upon seeing the arena walls the rats were possibly motivated to explore them despite the noise. Another reason why Hayman et al. argue for a stronger effect of the noise boundaries than I do could be that their data do not contain more than eight trials per rat, whereas mine contain 16 trials per rat. As shown in the results section I observed a higher occupancy in the noisy zone in later trials compared to early trials, i.e. the animals adapted to the noise.

As one of the two subjects showed significant adaptation while the other one showed a significant reduction of activity, experiments in virtual environments with noise boundaries should be designed to contain as few trials as possible. Additionally, mechanisms could be implemented to counteract disadvantageous long-term reactions, such as intensifying sound pressure as a function of distance from the silent zone and rewards for running in the silent zone.

3 Experiment 2 - Differentiating between Cue and Place Learning

3.1 Motivation

As outlined in previous sections, the application of noise boundaries led to a strong place preference for the silent zone. How can the results be explained? The experiment described in the following section will try to differentiate between two hypotheses:

- The underlying mechanism is a simple stimulus-response scheme. The rats have learned to associate the landmark with the noise switching off and approached it whenever the noise became intolerable (beacon following). Such a mechanism explains the observed behaviour without assuming any knowledge on spatial relationships.

- The fuzzy boundary arena induced place learning (compare section 1.2). The rat kept track of the position of the silent zone by performing path integration or it was able to deduce the zone's position with the help of distal landmarks.

In order to determine the kind of navigational mechanism employed by the rats I investigated the behaviour of one rat in a derivative of the fuzzy boundary arena which did not contain any local landmarks (termed "static boundary arena").

3.2 Materials and Methods

3.2.1 Subjects

One male Long–Evans rat *Rattus norvegicus* (Charles River, Germany), weighing 130-150g at the beginning of the experiments served as subject (rat 157). The subject was naive to the paradigm. All experiments were licensed according to German and EU law regulating the use of animals in research.

3.2.2 Experimental Room

The room was the same as in experiment 1.

3.2.3 Local Environment

In this paradigm there was only one, static noise boundary, crossing of which triggered the playback of aversive white noise (94dB). The boundary separated the silent zone, a square of 67cm x 67cm in the centre of the arena, from the noisy zone (remaining part of the arena). The silent zone was not cued by any landmark.

3.2.4 Tracking

As the analysis of this experiment required information about the rat's heading, two LEDs needed to be tracked. Because the subject had already received surgery (see section 4.2.5) I could use the two LEDs mounted on the headstage for tracking. The headstage was connected directly to the microdrive the animal had been implanted with (see figure 20). It was supplied with energy by a cable reaching down from the pre-amplifier located on the ceiling (compare figure 6).

3.2.5 Procedure

The procedure was the same as in experiment 1, except that no oat flakes were distributed in the arena because the rat did not need to be motivated to move.

One rat was tracked in ten trials in the static boundary arena. A trial lasted ten minutes. After ten trials the silent zone was shifted to a new position. Another three trials were run with the silent zone remaining at the new position. In ten control trials the rat moved in the same arena but no noise was ever played.

3.2.6 Analysis of Path Segments I - Straightness

In this experiment I investigated the straightness of path segments of excursions. An excursion began with the entry of the noisy zone (coming form the silent zone) and ended with the return to the silent zone. The aim was to compare the return segments to randomly chosen segments in order to find out if the return segments were particularly straight. I wrote a script which found an excursion and cut out the last part as well as a random part of equal length which did not overlap with the last part (the exact length was an input parameter to the algorithm). Next, it computed the cumulative, absolute change in heading along the last part and along the random segment and stored the two values. The algorithm continued with this procedure until no further excursions could be found. In the end there were two distributions of heading changes which could be compared with standard statistical tests. As the result of such a test depends on the random choice of segments, I performed repeated tests, comparing the return segments to a different sample of random segments each time. The significance threshold was Bonferroni-corrected to compensate for multiple comparisons. Apart from the absolute heading changes the script also compared the sinousity of the segments in both distributions (actual path length/length of straight path) as a control.

3.2.7 Analysis of Path Segments II - Alignment to Noise Boundaries

In addition to path straightness I assessed the angles of path segments in the silent zone at the nearest noise boundary. The aim of the analysis was to find out whether the rat showed

a tendency to move along the noise boundaries inside the silent zone, as it is the case with physical walls. Such a behaviour would result in many segments that are approximately parallel the nearest noise boundary (one would obtain mostly small angles). The expectation was that angles tend to be smaller in the test than in the control trials, when no noise was played.

To compare segment angles in the test and the control condition I wrote a Matlab script that segmented the silent zone into 64 bins of 8.4cm x 8.4cm. Whenever the rat passed through a bin the script computed a vector from the entry to the exit point and stored it (see figure 12). After having computed all vectors the script calculated an average vector for each bin. The angle of an average vector was the weighted average of the angles of all vectors falling into that bin. The weights were given by the vector lengths so that longer passages through a bin contributed stronger to the angle of the average vector than shorter passages. For convenience the sum of the weights was normalized to one.

Angles were always measured with respect to the nearest noise boundary and the vectors were deprived of directionality. Thus, only angles between 0° and 90° could occur. Practically, the procedure divided the bins into two populations: One in which the angle with the horizontal was measured and one in which the angle with the vertical was measured. As a result, an angle of 0° can always be interpreted as being parallel to the nearest boundary. Another consequence is that for bins along the diagonals an average vector cannot be computed because there is no nearest noise boundary.
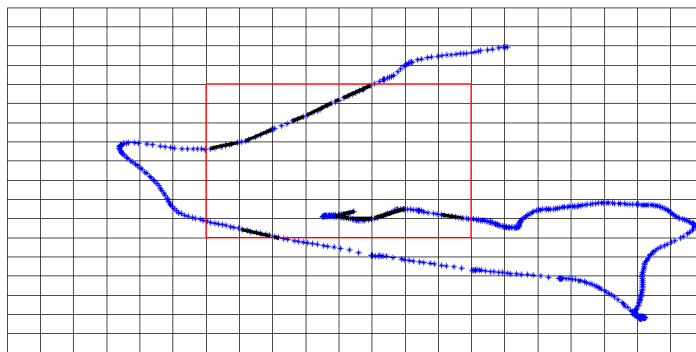

Figure 12: **An example of how the path was segmented by the algorithm. The blue dots show the position samples recorded by the tracking system. The bins are represented by the gray grid and the noise boundaries are shown in red. When the rat passed through a bin in the silent zone which did not lie on one of the diagonals the script computed the vector from the entry to the exit point (black lines). Note that the segmented path is a rather precise approximation of the true path due to the small bin width.**

The script produced so-called quiver plots, showing the average vectors for all bins. In the quiver plots shown in the results section the length of the vectors is inversely proportional to the standard deviation of all the angles contributing to the average angle. If the average vector in bin x was much smaller than in bin y that would mean that the variance of angles was much bigger in bin x.

3.3 Results

3.3.1 Raw Data

Inspection of the raw data suggested that the behaviour in this paradigm was similar to the behaviour in the fuzzy boundary arena (see figure 13). The rat mostly stayed inside the silent zone in early trials and made more visits to the noisy zone in later trials. In the control trials the rat mostly moved alongside the walls of the arena showing no particular interest in the silent zone.
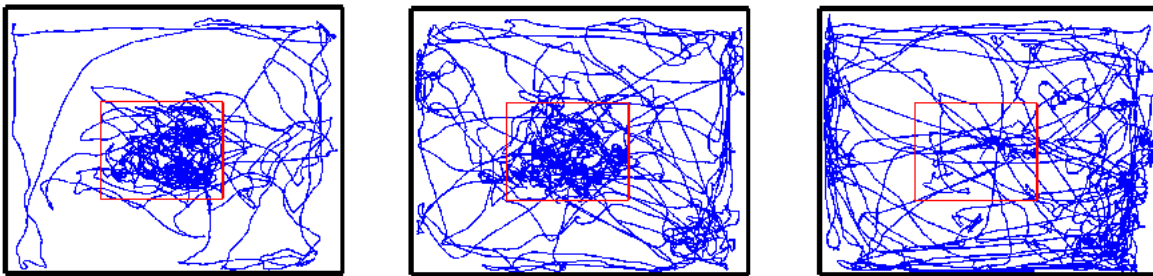

Figure 13: **Three selected trials. The path of the rat is shown in blue, the arena walls in black and the static noise boundary in red. On the first trial (left) the rat made fewer visits to the noisy zone than in the ninth trial (middle). In the control trials it mostly moved along the arena walls (right).**

3.3.2 Occupancy and Activity

As in the fuzzy boundary arena, the application of noise boundaries introduced a shift in occupancy (figure 14). In the control trials the rat spent little time in the silent zone (approx. 10% of the total time). When the noise boundaries were applied the relative occupancy in the silent zone increased dramatically (to approx. 60%). A similar shift but in the opposite direction was observed for the relative activity in the silent zone (from approx. 65% to 25%).
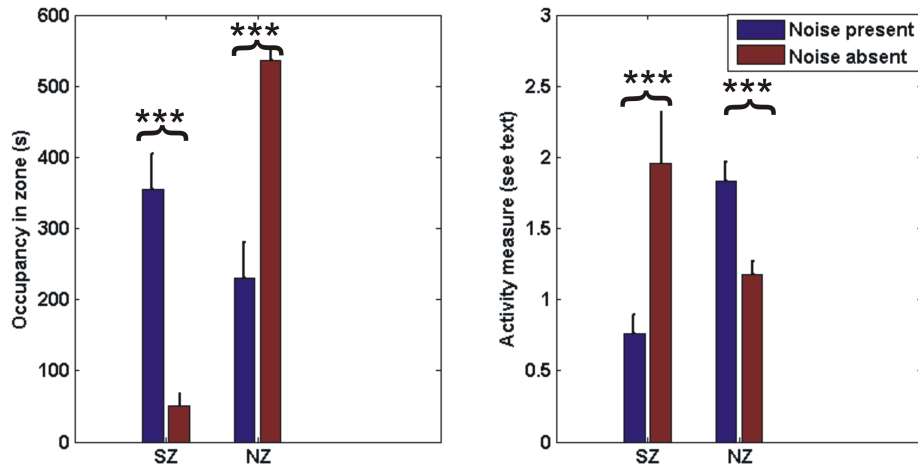
Figure 14: **Average occupancy and average activity in the silent zone (SZ) and the noisy zone (NZ). The errorbars indicate the SEM. Values obtained when the noise boundaries were present are depicted in blue, values from the control condition are displayed in red. P-values are symbolized by stars:** $* \Longrightarrow p < 0.05, ** \Longrightarrow p < 0.01, *** \Longrightarrow p < 0.001$**.**

3.3.3 Straightness of Return Paths to the Silent Zone

The fact that the landmark is not critical for the effectiveness of the paradigm speaks in favour of place learning. However, a stimulus-response association cannot be ruled out yet. It could be that the animal simply turned by 180° whenever the noise set in. If this was the case one would expect many short visits to the noisy zone.

The path taken in the time between the entry of the noisy zone and the reentry of the silent zone is henceforth referred to as an excursion. Figure 15 shows the lengths of all excursions the subject undertook in the ten trials.
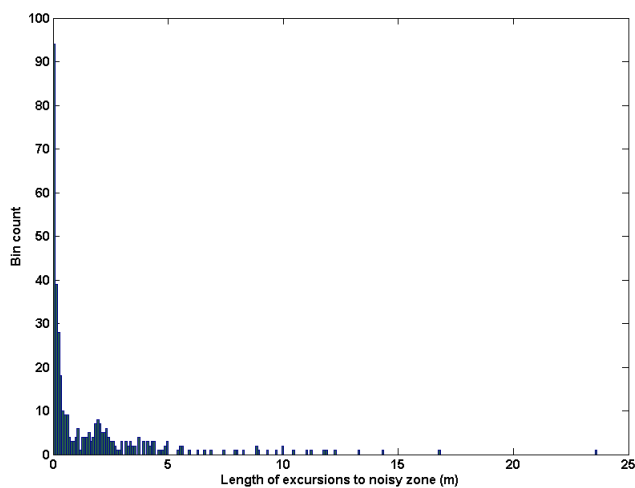

Figure 15: **Histogram of excursion lengths. The majority of excursions was very short.**

Indeed, the majority of excursions was very short. More than 50% of them were shorter than

0.5m (189 out of 375). However, there was also a substantial number of longer excursions, ranging up to 24m. Excursions that long neccessarily involve multiple changes of heading because straight movement is soon impeded by the arena walls. If the rat was indeed entirely uninformed about the position of the silent zone it must have found it by chance at the end of longer excursions. However, observation of the rat's behaviour suggested the opposite. The rat seemed to head back to the silent zone on a straight path after spending some time in the noisy zone.

Analysis of excursion segments verified that impression. In the last parts of the excursions (return segments) the rat made on average fewer heading changes than in randomly chosen excursion parts (random segments) stemming from the same excursions (the random segments were always chosen such that they had the same length as the return segments and did not overlap; for a description of the analysis see section 3.2.6). This statement was shown to be valid independent of the choice of random segments and independent of the exact segment length. Figure 16 shows the last 0.5m of all excursions made and one sample of random segments. Changing the segment length to a value other than 0.5m did not make a qualitative difference (see table 1). However, the difference between the return and the random segments became less obvious when the segment length approached 0.8m. This finding is probably due to the limited room available for excursions: The longer the segment length chosen for the analysis the higher the probability that the return segments include more path than the true return path.
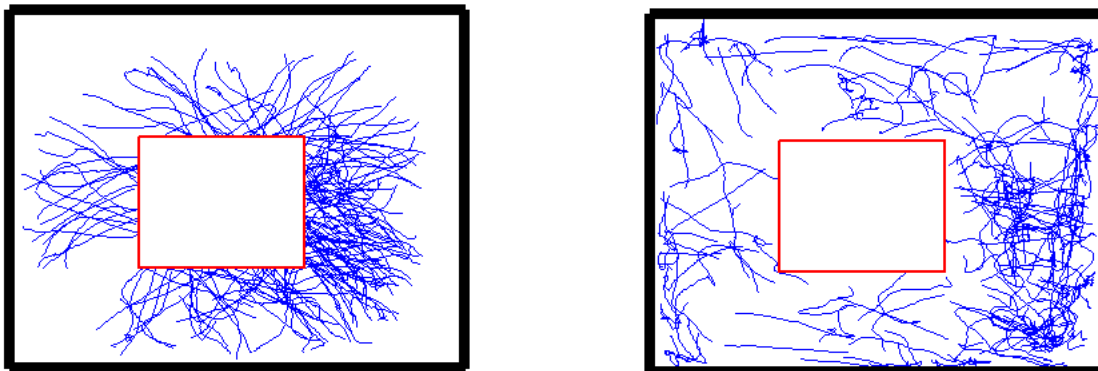

Figure 16: **Left: The last 0.5m (return segments) from all excursions longer than 1m. Right: Random sample of 0.5m segments of the same excursions, non-overlapping with the return segments. The return segments were compared to a hundred different of such random samples.**

Length of Segment (m)	Percentage
0.1	100
0.2	98
0.3	97
0.4	99
0.5	97
0.6	98
0.7	97
0.8	78

Table 1: **Percentage of comparisons in which the mean heading change was significantly smaller in the return than in the random segments for different segment lengths. In most comparisons the mean heading change was significantly smaller along the return segments than along the randomly chosen segments. Varying the length of the segments did not make a qualitative difference.**

3.3.4   Position Shift of the Silent Zone

Another indication for place learning was observed when the silent zone was shifted to a different position after ten trials. In the first minutes of the 11th trial the subject searched for the silent zone at the position where it used to be in the previous ten trials (see figure 17). After approximately three minutes the rat stopped searching at the old location and it did not reengage in the search in the following trials.

Interestingly, an increase of occupancy in the noisy zone in the shift trial was not observed, although the rat spent the first minutes almost exclusively in the noisy zone (see figure 17). Once the animal had found the silent zone it showed a stronger tendency to remain there than in previous trials. As in the first experiment, the effect of trial number on occupancy in the noisy zone was significant ($r = 13.9, CI = [3.8, 24.1], p = 0.011$). The shift interfered with adaptation only for one trial. Adaptation continued throughout the following two trials, in which the silent zone remained constant at the new position.
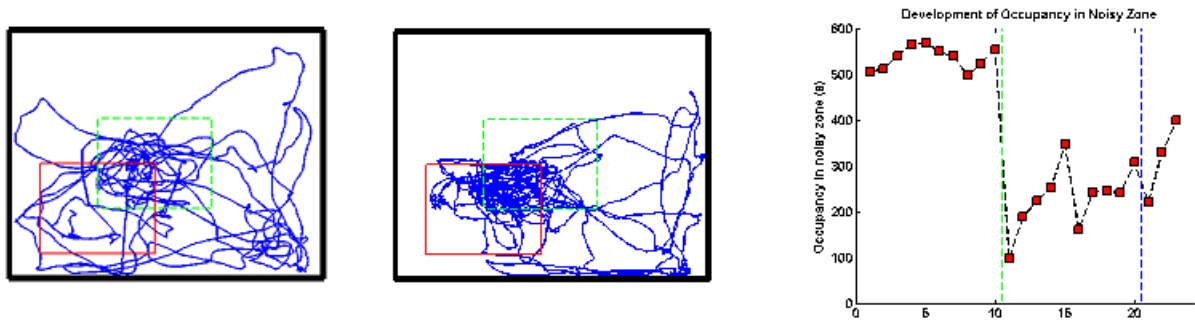
Figure 17: **Left: The first 3 minutes of the 11th trial, in which the silent zone was located at a new position. The green, dotted line marks the old position of the silent zone, the red line marks the new position. Note that the subject moved a lot in the area where the silent zone used to be in previous trials. Centre: The last seven minutes of 11th trial. The animal mostly stayed inside the silent zone, predominantly in the area which the old and the new silent zone had in common. Right: Occupancy in the noisy zone for all trials (horizontal axis: trial number). The green line separates the control trials (first ten data points) from the test trials. The blue line indicates the shift of the silent zone to a new position. Note that occupancy decreased just after the shift.**

3.3.5   Alignment of Paths to the Noise Boundaries

The fact that the rat returned to the silent zone on a straight path after long excursions and the observation of search behaviour after the shift of the silent zone suggests that the subject had learned where to find the silent zone in the arena. I wondered whether it had learned the exact spatial layout of the silent zone or simply its approximate location. In an attempt to find indications for the former hypothesis I investigated whether the animal showed a tendency to move in parallel to the noise boundaries inside the silent zone. As pointed out earlier, moving along the arena walls was a behaviour typically observed in the control trials. If the subject displayed the same tendency when moving inside the silent zone this would indicate that it had learned the exact spatial layout of the zones.

I divided the silent zone into multiple square bins and asked whether the passages through each bin tended to be more aligned to the noise boundaries in the test than in the control trials. To that end I computed an average vector for each bin reflecting the average orientation of the passages, ignoring directions. Figure 18 shows the average vectors for the control and for the test trials. Almost all of the average vectors in the control condition subtended an angle of about $45°$ at the nearest noise boundary. This is to be expected in case there is no preferred orientation. When the noise boundaries were applied the average vectors hardly changed. They did not tend to be orientated in parallel to the noise boundaries. The impression was confirmed by a Wilcoxon rank-sum test: The angles of the average vectors at the nearest noise boundary in the test condition were not significantly different from the angles in the control condition (p=0.08).
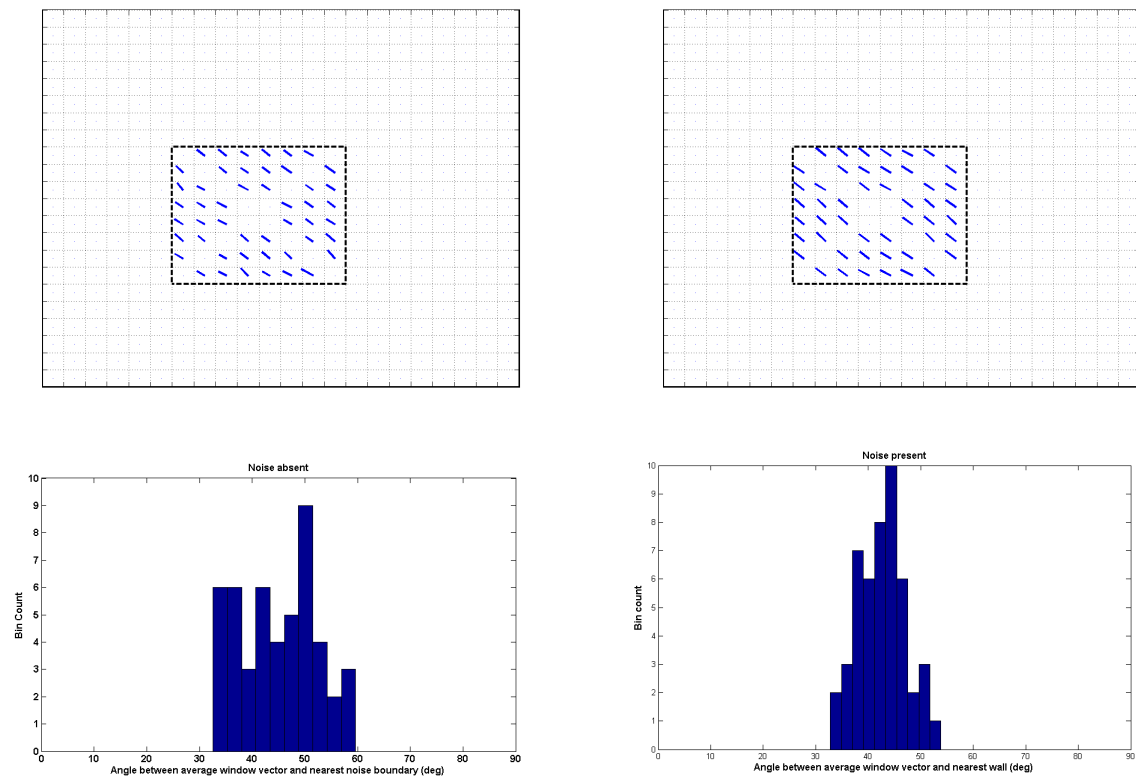
Figure 18: **Average angles between path segments and the noise boundaries were analyzed in order to find out whether the subject tended to move in parallel to the noise boundaries. If so, the angles subtended by the average vectors at the nearest noise boundary in the quiver plots should be smaller in the test (upper right) than in the control trials (upper left). The bins are represented by the grid and the silent zone is indicated by the dotted line. The average vectors for each bin in the silent zone are shown as blue lines. Note that the average vectors mostly subtend an angle of about 45° at the noise boundaries, regardless of the condition. The distribution of angles at the nearest noise boundary in the control condition (lower left) was not significantly different from the distribution in the test condition (lower right). The length of the average vectors in the upper plots is inversely proportional to the standard deviation of the contributing angles, i.e. the angles of the individual bin passages at the nearest noise boundary. Because the lengths do not differ between conditions one can conclude that the application of the noise boundaries did not lead to the emergence of a preferred orientation, not even on the level of individual bins.**

3.4 Discussion

Removing the local landmark cuing the silent zone and using a static rather than a fuzzy boundary did not reduce the effectiveness of the paradigm. In the control trials the mean relative occupancy in the silent zone was 10%. It increased to 60% when the noise boundaries were applied. Thus, the effect was as strong as in the fuzzy boundary arena. There the occupancy increased by 25% (subject 1) and 50%, respectively (subject 2). Therefore, I conclude that neither the landmark nor the fuzzy boundary are neccessary to induce a preference for the silent zone.

To better compare the fuzzy and the static noise boundary arena it would be more convenient to measure the same animal in both paradigms rather than choosing a between-subjects design. However, I could not use the animals from the previous experiment because they had adapted to the noise. Adaptation was also observed in the static boundary arena.

The obtained results disprove the hypothesis that a stimulus-response scheme can account for the behaviour in the static boundary arena. Because the removal of the local landmark did not make a noticeable difference beacon following cannot be the mechanism underlying the observed place preference. The hypothesis that the onset of noise triggered turning by 180° can be ruled out because it cannot explain why the rats returned to the silent zone on a comparably straight path after long excursions. Furthermore, it cannot explain why the animal changed its behaviour when the silent zone was shifted to a new location.

In summary, the results can be explained best by the concept of place learning. The rat must have been informed on the location of the silent zone with respect to stable landmarks such as the corners of the arena. After the shift the relationship between the silent zone and these landmarks had to be relearned.

As outlined in the introduction, finding uncued places might also be achieved by path integration. In this case path integration is not a good candidate to explain how the animals found the silent zone. If it was the predominant strategy shifting the silent zone to a new location between trials should have no effect. The animal was placed in the centre of the silent zone at the start of the shift trial. If it had integrated its movements from there it should have found the silent zone with the same precision as in all other trials. Moreover, the search behaviour observed after the shift points to the usage of long-term memory rather than path integration. However, a mixture of path integration and distal landmark use cannot be excluded.

The results suggest that the subject had learned the position of the silent zone. However, the precision of the acquired knowledge on spatial relationships remains unclear. Analysis of path alignment to the noise boundaries did not provide any indication that the animal tended to move alongside the noise boundaries inside the silent zone, the way it was observed for physical walls. Of course, this result cannot serve as evidence that the precise position of the noise boundaries had not been learned. It could be that the exact position had been learned but the information was not used to navigate alongside the noise boundaries. Maybe the usage of a more aversive

stimulus, such as electric foot shock, would be more effective in enforcing avoidance behaviour than noise.

4 Experiment 3 - Place Cell Responses to Noise Boundary Shift

4.1 Motivation

On the behavioural level I could not find indications that the rat had learned the precise position of the noise boundary. In order to find out whether rats are able to form a mental representation of the static boundary arena, including the noise boundaries, I performed extracellular recordings in the hippocampus while rats moved through the arena. As outlined in the introduction, the hippocampus was proposed to encode an allocentric representation of space which is used for navigation, the so-called cognitive map (O'Keefe and Nadel, 1978). If noise boundaries can be incorporated into the cognitive map, one would expect to observe changes in place cell activity in response to changes in the noise boundary layout.

I recorded in the same arena as in experiment 2 and examined the changes in place cell activity when the silent zone was scaled by a factor of two. I expected to obtain similar results like Burgess and O'Keefe who made the same experiment in rooms defined by real walls (O'Keefe and Burgess, 1996). They found that place fields kept a fixed distance to a wall or a fixed ratio of distances between two opposing walls when the room was scaled up.

4.2 Materials and Methods

Place cell isolation was successful only in three subjects (rats 1, 4 and 694) while no place cells could be isolated from the animals 154, 156 and 157 . The following sections relate to the former.

4.2.1 Subjects

Three male Long–Evans rats *Rattus norvegicus* (Charles River, Germany), weighing 130-150g at the beginning of the experiments served as subjects. None of these took part in the experiments described earlier. In one of the animals I recorded from CA3 (rat 694) in the other two I recorded from CA1 (rats 1 and 4). All experiments were licensed according to German and EU law regulating the use of animals in research.

4.2.2 Experimental Room

The room was the same as in experiment 1 and 2.

4.2.3   Local Environment

As in experiment 2, there was only one, static noise boundary, crossing of which triggered the playback of aversive white noise (94dB). The silent zone was a square centered in the arena. In contrast to experiment 2, the size of the silent zone was varied in between trials (but was stable during a trial). It was either a 67cm x 67cm or 134cm x 134cm square. The setup did not contain any local landmarks.

4.2.4   Tracking

Tracking was performed the same way as in experiment 2.

4.2.5   Surgery

The animals were anesthetized by injecting 1 ml/kg ketamine (Ketamin Gräub, Albrecht, Aulendorf, Germany) and 0.25 ml/kg xylasel (Rompun, Bayer, Leverkusen, Germany) intraperitoneally. In order to test whether anaesthesia was sufficient the rat was pinched between the toes of a hindpaw with forceps. If the leg was withdrawn ("toe-pinch-reflex") more anaesthetic was administered (50% of the previous dose). When the reflex was no longer observable the rat's head was shaved and fixated in a stereotactile apparatus. During the entire surgery and during the recovery from anaesthesia the rat was lying on a heated underlay which kept a temperature of about 37 °C. The eyes were covered with salve (Bepanthen, Bayer) to avoid dehydration.

In the first step, the scalp was cut open and the positions lambda and bregma (defined as intersections between bone fissures; see figure 19) were identified by eye. A hole was drilled into the skull with a diameter of 2.3mm either 3.4mm posterior and 1.6mm lateral (targeting CA1) or 3.4mm posterior and 3mm lateral (targeting CA3) to bregma. The dura was removed with a fine cannula. The microdrive was positioned above the hole with a micro-manipulator and the tetodes were inserted into the tissue. For insertions into CA1 the tetrodes were pushed 1.5mm into the tissue, for insertions into CA3 they were pushed 2.2mm. Four additional holes with a diameter of 1.2mm were drilled into the skull at arbitrary positions and a fixation screw was screwed into each hole. One of these served as ground. In the next step the leading cannulas of the microdrive were embedded in a protective layer of Vaseline (Vaselin, Caesar & Loretz, Hilden). Then cement (Paladur, Heraeus Kulzer, Hanau) was distributed all over the exposed skull. It covered the lower parts of the metal legs of the microdrive. Thus, the cement held the microdrive and the fixation screws served as mechanical link between the skull and the layer of cement.

Immediately after surgery 2 ml sodium-chloride solution (0.9%) were injected into the neck subcutaneously to avoid dehydration. In the first hours after surgery the animal was provided with water but not with food and the water uptake was monitored. In the first two days after

surgery the animal was injected with 1 ml/kg Rimadyl (1:10 dilution) twice a day for pain treatment.
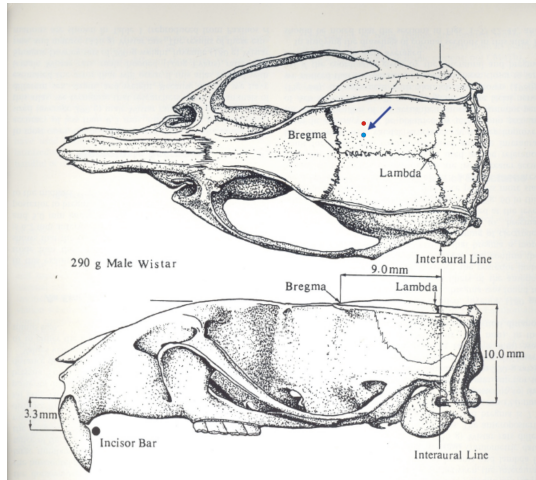

Figure 19: **Dorsal and lateral view on a rat skull. The points lambda and bregma served as reference for the electrode placement. The entry points of the tetrodes are indicated as circles (CA3: red, CA1: blue). Taken from Paxinos and Watson 1998.**

4.2.6   Spike Recording

Neuronal potentials were recorded with twisted-wire nickel/chrome tetrodes (80% nickel, 20% chrome, A-M Systems, Carlsborg, USA). The tetrodes were part of a chronic implant called microdrive (see figure 20). The microdrive contained four screws which allowed to move each tetrode up and down individually. One full turn of a screw moved a tetrode $250\mu m$. Tetrodes and microdrives were made in our laboratory (for a detailed construction guidance see Jovalekic 2008 and Libchik 2008).
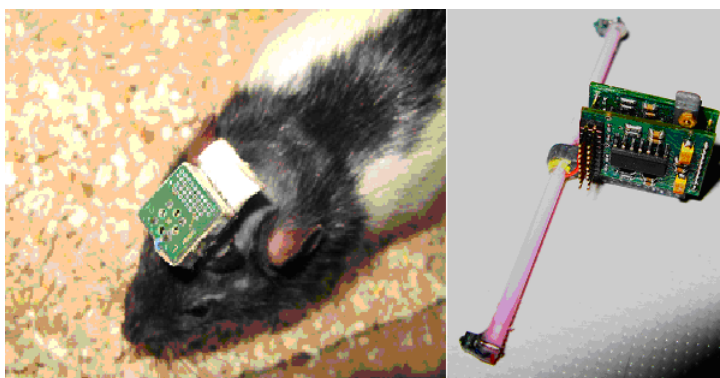

Figure 20: **Potentials were picked up by four tetrodes which could be moved individually via screws in the micordrive implant (left). The tetrodes fed the signal into the headstage (right) which facilitated impedence conversion. It carried two infrared LEDs for tracking.**

Spike recording was facilitated by the hard- and software system Dacq (Axona, St. Albans, UK). The following section will briefly describe the elements of the recording system. For a

detailed description I refer to the dacq user guide.

The signal was fed from the microdrive to the headstage, a small electronic device which could be connected directly to the microdrive and facilitated impedance conversion (see figure 20). The headstage was connected to a pre-amplifier (gain: 1000) by a cable of 3m length. Following amplification each signal was split into a differential pair for further transfer to the main recording system. The splitting allowed to transfer the signal up to 20m with minimal risk of noise pickup. In the main recording unit the signal was further amplified (adjustable gain between 0.25 and 64) and high-pass filtered (3dB cutoff 360Hz). Taking both amplifiers into account the signal could be amplified by a factor ranging between 250 and 64000. The gain chosen for this study varied between 8000 and 50 000 depending on the strength of the original signal.

The system only stored the signal from a particular tetrode if the voltage exceeded an experimenter-defined threshold on any of the four channels of that tetrode. The threshold could be adjusted manually in the digital oscilloscope display included in the recording software. On threshold crossing the voltage traces on all four channels were stored (time window from $200\mu s$ pre- to $800\mu s$ post-threshold).

4.2.7 Cluster Analysis and Analysis of Spatial Selectivity

Clustering was performed with the program Tint (Axona, St. Albans, UK). The software allowed to assess a number of waveform features such as peak or trough amplitude and displayed multiple 2-dimensional feature plots (e.g. peak amplitude on channel 1 versus peak amplitude on channel 2). In the feature plots every recorded spike was represented as a dot. In case a cluster could be identified by visual inspection the experimenter assigned all dots in that cluster to a cell by drawing a polygon around it with the mouse. Following the assignment an overlay of all waveforms in the cluster was displayed. Spikes were attributed to a cell only if they formed a well isolated cluster in at least one of the feature plots. All spikes in the cluster were required to be identical in shape, i.e. to have the same four-channel-profile. In case the waveforms were identical on all four channels the spikes were considered artifacts. Furthermore, the rising part of the waveforms was required to be steeper than the falling part (as depolarisation is faster than hyperpolarisation in pyramidal neurons).

Once spikes had been attributed to a cell it was analyzed whether that cell increased its firing rate drastically at a specific place. For this purpose Tint computed so-called rate maps. Rate maps are histograms in which the axes represent 2-dimensional space and the firing rate is colour-coded. Cold colours signify low firing rates and warm colours signify high firing rates. A cell was considered a place cell if it increased its firing rate whenever the rat was at or near one particular location (place field). The place field was required to be a Gaussian in colour code. The peak rate needed to exceed 1Hz. Cells with multiple place fields were also accepted, as long as the number of fields was smaller than 4.

In addition to rate maps the program could display the path of the rat and superimpose the spikes of a particular cell at the positions they occurred. This form of display was used to exclude artifacts. In case most or all of the spikes occurred during a single passage through the putative place field they were considered to be artifacts.

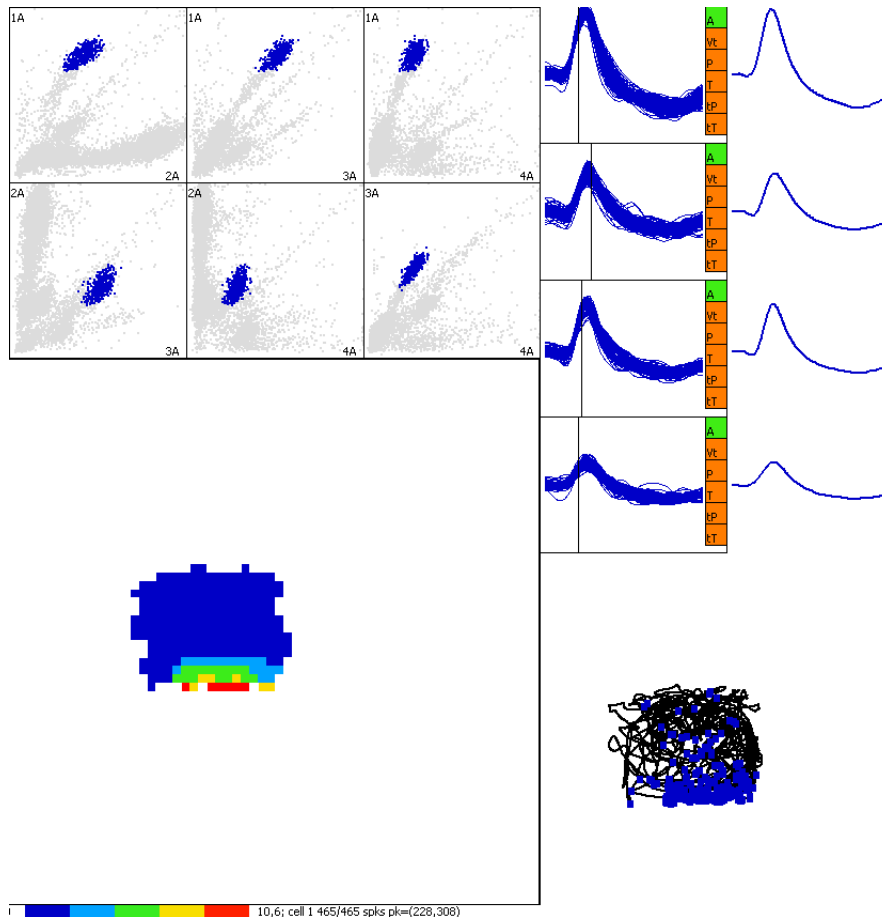Figure 21 shows the feature plots, the waveform overlay and the rate map for a typical place cell.

Figure 21: **The software tools used to identify place cells. Clusters were identified by visual inspection in the Tint cluster cutting window (upper left). The dark dots represent spikes that were assigned to a cluster (axes: peak-trough amplitude). An overlay of all spikes in that cluster can be seen in the upper right. Note that all spikes are identical in shape. One would therefore assume that all spikes in that cluster originate from the same cell. The rate map for that cell is shown in the lower left. The cell increased its firing rate whenever the rat's head was at or close to the central part of the south wall. The legend underneath shows the peak firing rate (10.6 Hz) and the colour code. Colours correspond to percentages of the peak firing rate. For example, in the green regions the firing rate was bigger than $2/5*10.6Hz$ and smaller than $3/5*10.6Hz$. To the right of the colour legend there is the cell number, the number of spikes contributing to the map divided by the total number of spikes assigned to the cell and the coordinates of the place field peak. In the plot in the lower right the path of the rat is shown, including all the spikes fired by the cell (blue dots). The cell was almost inactive outside the place field.**

4.2.8 Procedure

Every workday morning and afternoon screening trials were run with each subject in a wooden box of 50cm x 40cm area, lined with cellulose sheets. A screening trial lasted eight minutes. During screening trials the rats moved inside the box freely while extracellular unit activity was recorded. If the recordings did not contain place cell activity the tetrodes were driven approximately $60\mu m$ deeper into the tissue (1/4 turn). If posthoc analysis revealed place cell activity recordings were undertaken in the arena.

A recording session in the arena consisted of three trials. In the first trial the size of the silent zone was 67cm x 67cm. It lasted 20 minutes. In case posthoc analysis revealed one or more units with a place field in the arena, the arena was wiped with ethanol and a second, identical trial was run. The second trial served to assess the stability of the place fields. In case a field was stable in the second trial a third trial was run.

In the third trial the silent zone was scaled up by a factor of two. It lasted 30 minutes. The third trial served to assess possible changes in place field position and shape in response to the scaling.

4.3 Results

In the screening trials 14 units could be isolated which qualified as place cells (seven in rat 1, four in rat 4 and three in rat 694). Eight of these units did have a stable place field in the box but not in the arena (see figure 22) and one unit stopped firing in the arena.
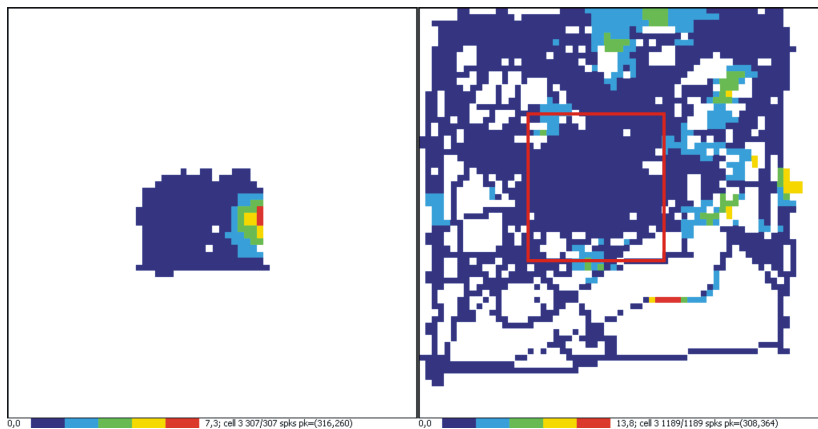

Figure 22: **An example of a unit which did have a place field in the screening box (left) but not in the arena (right). Firing rate is colour-coded, regions not entered by the rat appear white. The noise boundary is represented by the red square.**

Two units were recorded which did not react to the upscaling by remapping but one of these cells showed a reduction of the peak rate when the silent zone was scaled up (see figure 23).
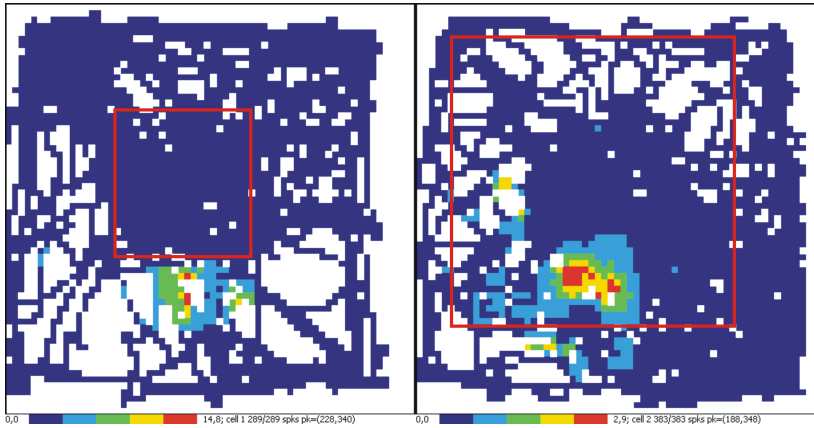
Figure 23: **An example of a field which did not remap in response to the upscaling of the silent zone. It remained stable at its position south of the arena centre (not true compass directions). Note, however, that the peak firing rate was reduced from 14.8Hz to 2.9Hz when the shift occurred.**

Two out of 13 units did have a stable field in the arena and showed remapping in response to the shift of the noise boundary. The field of one unit remapped to a completely new position each time the noise boundary changed (see figure 24(a)). Another unit developed a secondary field when the silent zone was scaled up (see figure 24(b)). The secondary field resembled a mirror image of the original field, the axis of reflection being the north-south axis through the centre of the arena. The response could be reproduced by down- and upscaling the silent zone a second time.

Finally, one unit was recorded which lost its spatial selectivity when the noise boundary was shifted to the periphery (see figure 24). The response was reproducible. However, in this case it is not clear whether the spatial distribution of spikes was really different in the two conditions. The apparent field in the first condition (small silent zone) might have arisen only because the rat did hardly enter the noisy zone.

Table 2 summarizes the electrophysiological results.
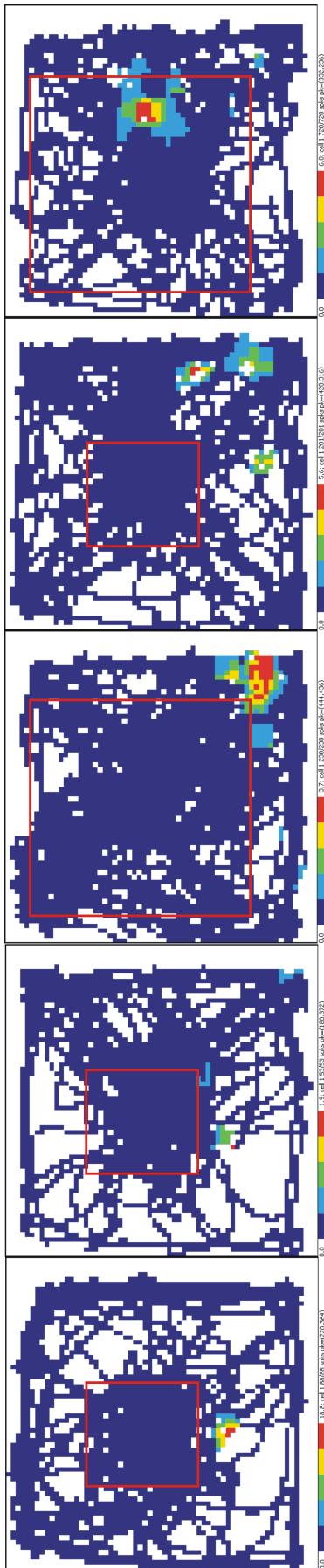
(a) **A field which remapped randomly when the noise boundaries changed. The figure shows five trials in the order they occurred. In the first and second trial the noise boundary remained constant and so did the field. In response to the first upscaling the field moved from its position south of the silent zone to the south-east corner of the arena. When the silent zone was downscaled the field split into three subfields, none of which resembled the field of the first two trials. When the noise boundary was shifted again the field remapped to the east, this time lying inside the silent zone.**
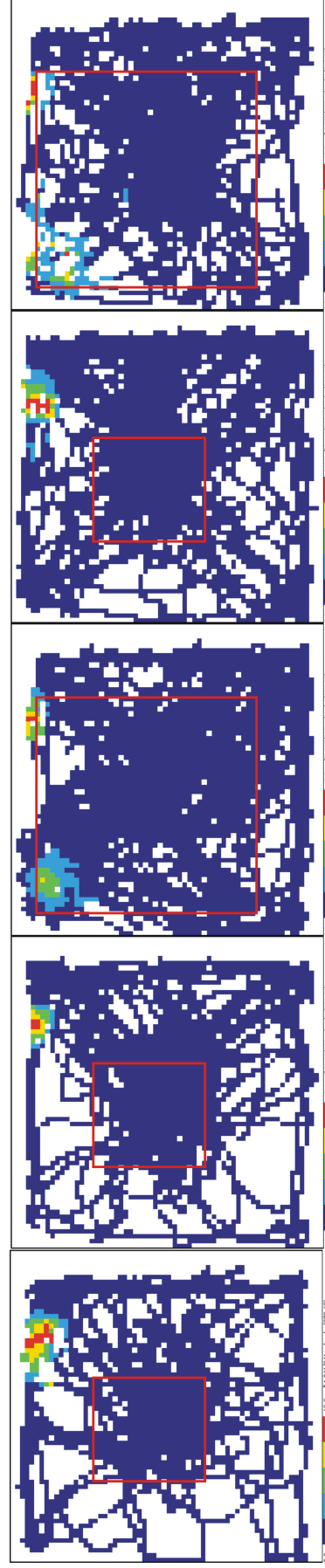
(b) **Field division in response to noise boundary shift. The figure shows five trials in the order they occurred. When the silent zone was small the unit had a field near the north-east corner of the arena. When the noise boundary cut the primary field and the unit developed a secondary field in the north-west corner.**
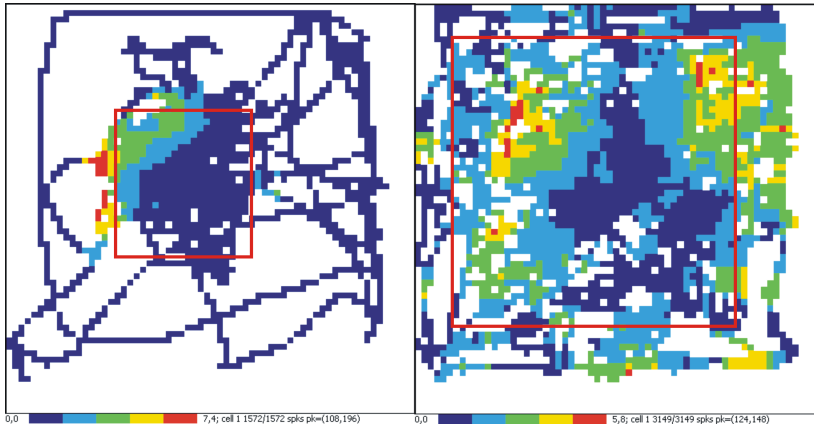
Figure 24: **Rate map of a unit which lost its spatial selectivity in response to the shift of the noise boundary. The field to the west of the silent zone disappeared when the zone was expanded. However, the spatial distribution of spikes may not have changed when the silent zone was scaled up. The different rate maps may be the result of the different spatial sampling.**

Cell	Subject	Tetrode	Field in Arena?	Response to Shift	Reproducible?
1		2	no	-	-
2		1	yes	loss of field	yes
3		3	no	-	-
4	1	2	no	-	-
5		2	no	-	-
6		2	no	-	-
7		2	no	-	-
8		4	yes	stable field	no
9	4	4	yes	stable field	no
10		4	yes	random remapping	-
11		4	yes	field division	yes
12		2	no	-	-
13	694	4	no	-	-
14		4	no	-	-

Table 2: **Overview of place cell responses.**

4.4 Discussion

Burgess and O'Keefe reported that the majority of place fields moved along when the (physical) boundaries in their setup were moved outwards (O'Keefe and Burgess, 1996). Cells with such fields convey information about the rat's position relative to the boundaries. In this experiment I did not find fields moving outwards with the noise boundaries. Thus, I could not provide evidence that the rats were informed about their own position relative to the noise boundaries. Interestingly, most of the place cells (nine out of 13) found in the screening did not have stable place fields in the arena. Some of the fields that were found (two out of five) did not remap when the noise boundary was moved. One may therefore speculate that noise boundaries are not as potent as physical boundaries in driving place cells, irrespective whether they are "fuzzy" or static.

However, one cannot say that the noise boundaries did not affect place cell activity. Three units responded to the change in noise boundary layout and in two cases the response could be reproduced. One cell remapped randomly, another lost its spatial selectivity and a third developed a secondary field. The latter two cases were also reported by Burgess and O'Keefe. Field division was of particular importance for their interpretation: They hypothesized that place fields are composed of independent subcomponents which can be pulled apart in larger environments (O'Keefe and Burgess, 1996). They proposed these subcomponents to be "tied at fixed distances to opposite walls". It is hard to see how this explanation could apply to the field presented here. The field divided when it was "squeezed" between the noise and the arena boundary and one subfield was identical to the original field.

In summary, the responses recorded in this study are at most suitable to signal that a change has occurred but not how the layout of the zones has changed in detail. Again, the absence of proof does not falsify the hypothesis. It is conceivable that those cells which signal position with respect to the noise boundaries were present but not found. The number of place cells that contributed to the analysis was rather small. Place cell isolation was not successful in the first set of subjects due to several hardware problems. The vulnerability of the microdrives was a major problem. In the second set of animals I did not reach the target depth in time with the majority of tetrodes so that only a few cells could be isolated.

It is well known that place cells can switch between different sources of input depending on the circumstances (Jeffery, 2007). It might be possible to increase the probability to find place fields following the noise boundary by repeating the experiment in total darkness. As visual information becomes unavailable the role of path integration might be strengthened. Path integration is a mechanism suitable for determining one's distance from an invisible boundary. The information coming from a neuronal path integrator should theoretically suffice to establish place fields which occur at a fixed distance from a noise boundary.

5   Conclusions

For this thesis I investigated behaviour in an experimental setup known as the fuzzy boundary arena (Hayman et al., 2008) and in a derivative of the setup, termed static boundary arena. In addition, I recored extracellular unit activity in the hippocampus in the latter environment. The obtained results allow three major conclusions:

1. Noise boundaries are useful to manipulate a rat's range in environments lacking physical boundaries. They do not perfectly constrain the animals to the silent zone but they induce a strong place preference.

2. The location of the silent zone in the arena can be learned by the rats. They are able to find it without having to rely on local landmarks.

3. Noise boundaries, unlike physical boundaries, are not potent determinants of place fields.

The fact that rats could learn the location of the silent zone, even though it was not directly marked, suggests that they have formed an internal representation of the arena and its zones. As some of the place cells isolated in this study reacted to a shift of the noise boundary it seems plausible that the hippocampus is involved in the formation of that representation. Thus, my results are - to some degree - in line with the cognitive map theory.

However, if the representation should really resemble a map, the map must be rather imprecise. I could not provide evidence for the hypothesis that the animals were informed about their distance from the noise boundary as they moved in the arena. Neither did they move along the noise boundary nor did I find place cells which had a place field at a fixed distance from the boundary. On the basis of these findings I suggest that the representation formed by the animals did not include precise metric information.

In similar experiments in rooms limited by physical walls Burgess and O'Keefe came to the opposite conclusion (O'Keefe and Burgess, 1996). They reported that place fields occur at fixed distances from boundaries. The conflict between the studies is probably due to the different stimuli employed in the experiments. Noise boundaries provide different sensory input than physical boundaries. They cannot be directly perceived except for the moment they are crossed. At all other times their position can only be inferred; either with the help of a perceivable landmark or of idiothetic signals (path integration).

It is conceivable that BVCs require more or other input than that to produce an output which eventually leads to place cell activity informative on the animal's position relative to the noise boundary. Thus, noise boundaries might not allow for metric representations of the environment by nature. The hypothesis can be assessed by investigating the responses of boundary cells in the entorhinal cortex to noise boundaries.

Importantly, the results suggest that an imprecise "map" can be formed even when the animal is not able to compute distances properly, i.e. that internal representations of space need not include metric information necessarily.

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