Rats in Virtual Reality: The Development of an Advanced Method to Study Animal Behaviour

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1 Introduction

1.1 The wild rat

Thousands of years ago, the Norway rat, which is the species almost all laboratory rats belong to, lived as wild rodent in northern China; however, little is known about its ecology and habits there. At some point, rats moved into the early human settlements. This relationship probably benefited the rats as the humans unwittingly provided them with food and kept their natural predators away. So the rat came to live with us in a human-dependent relationship (Krinke, 2000).
Today, rats are found just about everywhere humans reside. They live in cities, on farms, in subways and sewers all over the world. Except for northern China, where wild rats still inhabit burrows today, rats no longer live in the wild. Today the natural habitat of the wild Norway rat is the human settlement.
Calhoun (1963) kept wild rats in a semi-natural enclosure and studied their behaviour. He found that wild Norway rats dig and live in underground burrows. Burrows can consist of one single chamber connected to the outside by a short tunnel, or they can form a large complex of interconnecting tunnels, passages and cavities. Most of the time, they are built by a pregnant rat shortly before giving birth. The initial tunnel usually ends in a nest cavity. After a few days, the rat digs a second entrance from below. This second hole has no excavated dirt around it and is called a bolt hole; it may serve as an escape exit in case the burrow is invaded. Later expansions of the burrow system follow this same pattern of tunnel/cavity/bolt hole. Outside the burrow, rats tend to confine their movements to the same routes every day, therefore trails form gradually on the surface (Calhoun, 1963).
In the wild, rats are bound to a home range which is more ore less centred on their nest. Rats explore and navigate successfully in large areas. In a radio-tracking study, home ranges of the rat are reported to vary between 0.33 and $1.83 * 10^5$ m^2 and range lengths between 86 m and 311 m (Dowding and Murphy, 1994). In addition, much larger distances up to 954 m are reported for migrating single recaptured males (Hartley and Bishop, 1979).

1.2 The rat as an experimental subject

In some way the rat is an odd choice as a laboratory animal. Firstly, the rat is nocturnal, whereas most scientists are not, who, additionally, prefer to work in relatively bright light. Secondly, the rat lives naturally in burrows, as mentioned above, and in the crowded grassy ground environment that tops the burrow. A laboratory environment is the complete opposite of this natural environment, which is rich in sensual information: clean, bright and smooth. Irrespective of this, the rat serves as a very popular subject for biological and pharmaceutical science for several reasons. Firstly, rats are small and easy to house. In a compartment comparable in size to a common closet, 30-40 rats can be housed easily, whereas only one chimpanzee requires ten times more space. Secondly, the reproductive rate in rats is very high. A female rat is fertile 5-6 weeks after its birth. After fertilization, it is pregnant for 22-24 days and can give birth to 8-16 newborns. Only three weeks after delivery, the female can be fertilized again; this can lead to a maximum of eight generation cycles in one year, which can ideally result in up to 16^8 or 4,294.967,296 descendents from one female individual. Of

course, this number is only hypothetical since only 5 % of the new born survive the first year in free nature (Barnett, 1975). This fertility is directly connected with the third reason, namely that the generation cycle of rats is very short. Animals with short generation cycles are very suitable for breeding and the study of heredity since the effects or the outcome of a hybridisation can be observed within a few weeks. The high generation cycle also guarantees the supply of new animals with almost no delay. Finally, rats are very robust animals and do not require special technical efforts, as animals from tropical areas would do in the Western European climate. These benefits made them the number one experimental animal in the world and thus one of the best examined organisms known to men.

1.3 The sensual system of the rat

1.3.1 Somatosensation, vibrissae and olfaction

In contrast to humans, it is assumed that the most important sense of the rat is not the visual sense but the tactile sense of its vibrissae (see Fig. 1). Although this is just an assumption derived from the relative size of the cortical surface which processes the different sensory inputs, it is out of question that these areas are very prominent (Chapin and Lin, 1984). One can imagine that tactile and vibrissae senses become more prominent for a nocturnal animal since they are more reliable in the darkness. The visual sense, on the other hand, plays a minor role under these circumstances.

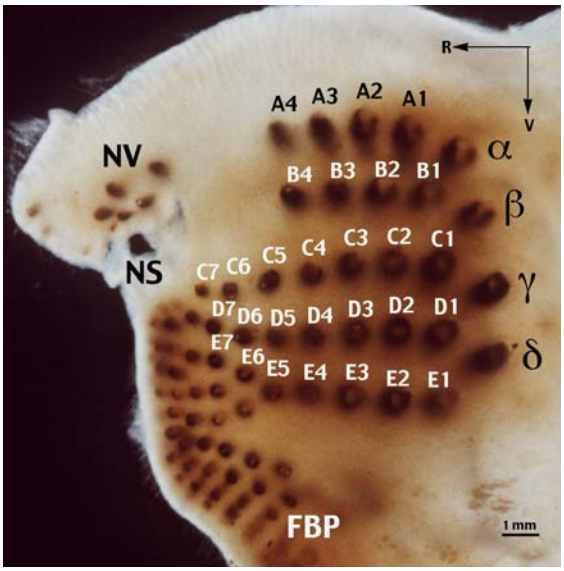


Fig. 1 The arrangement of the rat's vibrissae over the skin of the upper jaw. A-E represents rows in which the main vibrissae are arranged. They are numbered ascending from caudal to rostral. The four longest vibrissae lie between these rows and are marked α,β,γ,δ. The smaller secondary vibrissae are located in the furry buccal pad (FBP) and on the nose (nasal vibrissae NV; NS marks the position of the nostril). (Figure freely available from Barrels Web: http://www.neurobio.pitt.edu/barrels/pics.htm; courtesy of S. Haidarliu and E. Ahissar).

The rat's vibrissae system is especially remarkable; unlike the human tactile system it is not only a passive sensor, but, due to their constant movements, the animals are able to actively scan their environment. When scanning, the rat moves its whiskers forwards and backwards rhythmically with a frequency of 5-9 Hz (Carvell and Simons, 1990). During this movement, the rat sweeps the surrounding surfaces with the tips of its whiskers and receives tactical information from them. In discrimination experiments, rats have shown to reliably detect differences in the surface texture as small as 30 µm and spaced at 90 µm intervals (Carvell and Simons, 1990). This is comparable to primates using their finger tips, but still impressive if one keeps in mind that the rat is able to perceive this information over a distance of 45-60 mm, which corresponds to the length of the longest whiskers (Ibrahim and Whright, 1975).

The whisking behaviour is directly connected with sniffing, by which the olfactory sense supports the information derived from whisking. In addition to this senses, rats also use their front paws on which short whiskers are located, as well. This can be seen from the fact that they scan the same regions with their paws which the nose has swept only a moment before. Together, these systems give the animals a good impression of their close surrounding even in complete darkness.

1.3.2 Vision

The eye of the rat is designed for nocturnal life and therefore very light sensitive but poor in acuity. It lacks a fovea but contains rods and cones; these latter, however, represent only 1 % of the amount of photo detector cells (La Vail, 1976). Rats are typical mammalian dichromats with a short wavelength receptor which has its peak sensitivity frequency at 359 nm and a mid wavelength receptor with a peak sensitivity frequency of 510 nm. Beyond a frequency of 650 nm, very little sensitivity is left. There have been controversial discussions about the functionality of the rat's cone system; however, Jacobs et al (Jacobs et al., 2001) have shown that rats can perform colour discriminations. The acuity of the rat's eye measured by a discrimination task is 1.0-1.1 cycles per degree, which is much lower than the human acuity of 30 cycles by degree (Dean, 1978). Nonetheless, if one thinks of the fact that rats do not have a fovea, their acuity is fairly comparable to the acuity which can be found in the human peripheral retina.

Another feature of the rat's eye is its wide visual field, which results from the lateral position and the wide angel optics of the eyes. The figure from Hughes ophtalmoscopical measurement (Fig. 2) displays the outlines of the visual field for the right and left eye (Hughes,1979).

One can see that along the equator the visual field covers close to 360°. There is also wide area in which the two fields overlap and form a binocular field. To which extent the animals use this area for binocular vision is not known yet.

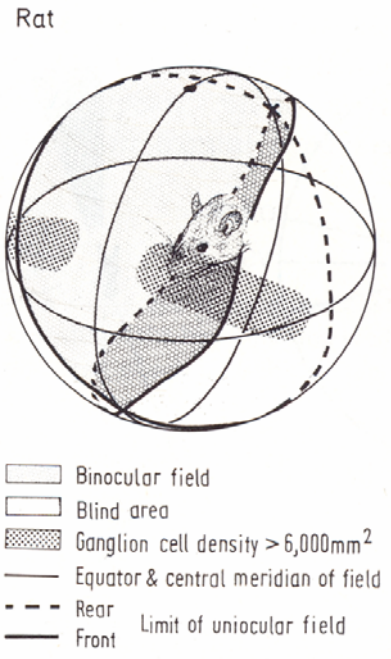


Fig. 2 Ophthalmosopically defined, absolute ocular fields of the rat. From Hughes (1976).

1.4. The cognitive basis of spatial behaviour

Beside in genetics, pharmacology etc., rats have also been used to investigate the properties and neuronal implementation of spatial behaviour. But not only rats were subject to this question; the huge variety of spatial behaviours which can be observed in nature range from

simple cue driven actions, for example in insects, to the most sophisticated cognitive processings in humans. To get an overview of this variety, such navigational abilities can be split into four levels requiring memory of different complexity and to a different extend. These levels include course stabilization, navigation by control rules, survey navigation and regions and non-spatial knowledge. To which amount rats are capable of these levels is still unclear and remains intensely discussed.

1.4.1 Course stabilization

Keeping a stable course in a cluttered environment is an important task in navigation which does not rely on a long-term memory of the spatial layout of that particular environment. In animals, course stabilization can best be studied in flying insects, for example in honey-bees. Here, optic flow is the crucial cue, e.g. for centring in a corridor, slowing down when passing through an aperture, or making grazing landings (Srinivasan et al., 1996). In humans, the use of optic flow for approaching a visual goal has been clearly demonstrated by Warren and co-workers using virtual environment technology. Observers were asked to approach a visible target but were displaced sideways during their approach. If they simply kept walking towards the goal, a curved trajectory would arise. If, however, subjects judged their actual egomotion by optic flow, they should keep a straight track by compensating the sideways displacement. Indeed, this is what happens if sufficient visual information for judging optic flow is presented. Concerning the speed of travel, Snowdon et al. (1998) showed that subjects who were asked to keep a constant speed in a driving simulator sped up if visibility was reduced by the simulation of fog. Conversely, if asked to match a speed level presented under different visibility conditions, subjects reduced speed in the samples presented with less visibility. Again, this is evidence for a simple, optic flow-based mechanism for judging egomotion. It is surely out of question that rats, like all higher mammals, are able to perform the simple behaviour of course stabilisation. The question if an occulomotoric coupling exists also in rats was a source of inspiration for one of our experiments (see Chapter 3.5).

1.4.2 Control rules and routes

The next level of navigational abilities described here requires long-term memory. In its simplest appearance, this can contain a snapshot image of the nest entrance which is used for image based homing, as it is described for bees (Cartwright and Collet, 1983). By minimizing the difference between stored and actual view, a starting point can be approached. In this case, only a simple view needs to be stored in the long-term memory and no higher-level image processing like for example recognition is required. A comparably small amount of memory is used for the storage of a homing vector in the path integrating mechanism of the cataglyphis ant (Wehner, 2003). On an outbound path from the nest, the animal integrates its travelled distance and turning angle, which results in a constantly updated vector that contains direction and distance to the starting point. Such an ability for path integration can also be observed in humans, but their acuity is much lower than the one of the ants. It is also interesting that humans use path integration only in situations where no other information is present or reliable (Foo et al., 2005). This publication was again the inspiration for one of our experiments (see Chapter 3.15). Following this hierarchy, one can imagine that if these mechanisms are used to calculate the way back to the nest, they are also used on the outbound path to find for example a feeder. Supposing that there are several feeders, the memory most likely contains several places and movement instructions associated with it. This should lead us then towards route memory in which those elements are arranged in a sequential order. In

general, routes are defined as places or views of places associated with movement instructions which lead towards the next place and so on (O´Keefe and Nadel, 1978). But to stay in line with the hierarchy, this definition must be examined a bit more closely. On a lower level the places are recognised, as described above, by a simple image matching mechanism. However, there are certainly more elaborate levels of routes in which the places are identified by object recognition of landmarks or by the configuration of several different landmarks. The ability of rats to navigate by using landmark information was proven, amongst others, with the help of the Morris water maze described in (Morris et al.,1982) and also inspired one of our experiments (see Chapters 3.11; 3.17). In a V.R navigation task with humans, Mallot and Gillner (2000) pointed out that humans follow a fairly robust recognition triggered response strategy. It turned out that movement decisions were associated directly with salient landmarks as changes in the periphery of the environment did not affect the performance.

1.4.3 Survey navigation

The term "cognitive map" was first introduced by Tolman (1948), who observed that rats could find novel shortcuts when navigating in complex mazes. This theory of a map-like representation of space in memory received much support by the finding that there are cells in the hippocampus which code for a definite place in the environment "place cells"(see Chapter 1.5). Along with the discussion about the location of a cognitive map in the brain, the question about the properties of these cognitive spatial representations arose. Beyond the route, the graph represents the next level in our hierarchy. In its simplest state, a graph consists of several places which are recognized by their view and is connected by movement instructions. A graph is, so to speak, a web of several interconnected routes. The sources which derive the connections between the places can implement different types of metrics into that representation: on the one hand, an egocentric metric which is observer centred and independent of global consistency and, on the other hand, an allocentric metric which is related to a global frame of reference and world centred (for details see Schölkopf and Mallot, 1995; Gillner and Mallot, 1998). Starting from a world centred graph, an increasing amount of details and metrical information finally leads to a topographic map which is embedded into a fully metric coordinate system. As mentioned above, it is still unclear and controversially discussed to which extent rats or other higher mammals are able to construct and use such a "cognitive map".

1.4.3 Regions and non-spatial information

Disregarding the still open discussion of content and complexity of the human spatial representation, some higher level spatial and non-spatial factors were investigated which have an influence on the representation of spatial content. One type of higher level factors is regionality. Humans tend to divide their known environment into regions, thus cities are often split into uptown and downtown, old town and new town or left of the river and right of the river. Borders of regions vary and can originate from natural elements, such as rivers; man-made, such as streets; or even virtual elements such as state borders. The finding that such regional information can distort the spatial representations of humans in direction judgements is evidence for the presence of these elements in human spatial representations (Hirtle and Jonides, 1985). Based on virtual reality experiments concerning this content, Wiener and Mallot (2004) formulated a fine to coarse route planning heuristic in which he states that humans prefer to choose routes which lead directly towards the target region and from there on in a more detailed fashion towards the target (Wiener and Mallot, 2004). Regional

information can also increase the learning performance in an unknown spatial environment. A comparable hypothesis is proposed by Kuipers (2003) in his skeleton model. He divided the environment not by regions, but by often used paths. The agent navigated from the start towards the next main path, i.e. skeleton, and, following this main path, into the target region and from there on to the destination itself (Kuipers et al., 2003). A model incorporating non-spatial factors is the world-graph of Lieblich and Arbib (1982), which includes some extra features into a graph model, as mentioned above. They introduced a current drive state of the animal, which can be hunger, fear, thirst, etc., which influences the decision making process where to move next. Another very common aspect of non-spatial factors which are widely spread in the animal kingdom is the intruder pressure that influences the spatial layout of a territory.

1.4.4 Other approaches

The hierarchy which is presented here is only one possibility of systematically arranging spatial behaviours. There are of course several other approaches to structure the variety of navigation mechanisms and spatial behaviours. As mentioned above, this hierarchy is structured according to the increasing complexity of memory required.
A fairly different approach might be a structure following a phylogenetic order. However, it is very hypothetical to make statements about the behavioural mechanisms of species which became extinct long ago. An approach which relies on more firm evidence is realised in an ontogenetically structured hierarchy. The development of the brain works hand in hand with the development of increasingly complex behaviours and the study of this development then allows us to draw conclusions about the development of the brain. Comparable to this is a hierarchy which is structured according to the acquisition sequence. Finally, one can order such a hierarchy with respect to the complexity of tasks, which takes the estimated computational effort for the navigation mechanisms into account.

1.5 The neuronal background of spatial behaviour

The knowledge about the brain regions and nuclei which are involved in the representation and computation of spatial behaviour is based on the results of two different branches of neurobiological science. On the one hand, knowledge derives from the spatial behaviour of humans and is limited to imaging or lesion studies. The other source of knowledge are animal studies of which rats and non human primates are the most common subjects. These studies are generally electrophysiological studies which measure the activity of single cells. Due to the difference in the subjects as well as in the methods, the results of these two groups are not always consistent. Imaging studies in humans, for example, have revealed a huge variety of brain regions which become activated during spatial tasks, for example occipitotemporal, parietal, and frontal cortical areas, as well as the hippocampus, the thalamus, and the striatum of the basal ganglia (Gazzaley et al., 2004). Animal research on this subject, on the other hand, was strongly influenced by the findings of O`Keefe and Dostrovsky (1971). By measuring the activity of cells in the hippocampus of rats while they where exploring an arena, they could show that the activity of some cells, which they called “place cells”, is correlated with the position of the animal in the arena. In humans, on the contrary, the role of the hippocampus was not that closely connected with spatial memory. A main influence on the functions of the hippocampus has been delivered by the publication of Scoville and Milner

(1957) in which they presented their work with a subject named H.M.. To cure his suffering from epileptic seizures, a brain surgery was performed during which parts of his medial temporal lobe, approximately two-thirds of his hippocampal formation, parahippocampal gyrus and amygdala were removed. The main effect of this surgery was that although his short term memory was intact, he was not able to commit new events to his long-term memory. However, his ability to form long-term procedural memories was still intact; thus he could, for example, learn new motor skills although he was not able to remember learning them. Based on these results, the role of the hippocampus was rather seen in the formation or mediation to long-term memory than in the representation of spatial contents. In later years, these seemingly contrary views reached a consensus. On the one hand, the establishment of spatial memory also requires the formation of a long term memory. Further tests with the patient H.M., which were focused on his spatial abilities, have revealed insights into the neural structures responsible for spatial memory and processing of spatial information (Corkin, 2002). On the other hand, the narrow focus of place cell studies have widened towards neighbouring areas of the hippocampus, where other interesting findings were made.
In the entorinal cortex, which serves as the main input region to the hippocampus, so-called "grid cells" could be found (Fyhn et al., 2004). These cells have spatially receptive fields which are arranged in a hexagonal grid. The information in a population of these cells can encode a certain place and therefore represents a precursor state of the information which is represented in the activity of a "place cell". A hypothetical model of this operation, which also includes the information from "head direction cells", is described in (Burgess et al., 2007). Another finding by Leutgeb et al. (2004) marks that different substructures of the hippocampus contain place cells which show different response characteristics. He reports that place cells in the hippocampal sublayer CA3 encoded individual environments, whereas place cells in CA1 showed significantly overlapping receptive fields in similar environments. This suggests that CA3 stores properties of individual environments, whereas CA1 contains a more category-like representation.
In humans, on the other hand, the role of the hippocampus for navigational purposes was also supported by the finding of Maguire et al. (2000). They compared the volume of the hippocampal formation between London taxi drivers and normal residents of London. After measuring this volume in a MRI scanner, they could show that the taxi drivers had a significantly bigger hippocampus than the control group.
From this point of view, is appears as if the hippocampus plays an important role in spatial behaviour, but rather as a mediatory instance towards higher associative areas than as the location of the cognitive map, as it was assumed after the first "place cell" findings. To which extend this higher areas are used, especially if one compares rats and humans, is still an open question.

1.6 VR as a method to investigate spatial behaviour

Since the early 1980s, computer and software technique improved exponentially. At the same time, virtual reality technique evolved into an important and powerful tool in science, medicine, military and throughout various domains of the computer business. While the strongest impulses might have come from the military, which has not only a big interest in simulator technique, but also the funding to afford expensive hardware and the necessary manpower to develop virtual reality applications, the key feature which derived virtual reality out of simple 3D computer graphic was, in my opinion, the invention of the "head mounted display" (HMD). Instead of simply watching something spatial on a screen, one now has the

illusion of being inside the 3D graphic or the virtual environment. The big step from shifting your gaze direction on a screen to actively looking around with eye, head and body movement brought the “virtual” closer to “reality”. It soon turned out that for perceiving a virtual environment as realistic, more than just wearing a HMD was necessary. The term ‘immersion’ started to play a role. But immersion is a subject hard to define. Roughly, one can say that it describes the intensity of the feeling how realistic the simulation is.
The most realistic virtual realities can probably be experienced in a modern simulator for air planes or comparable vehicles. With a huge technical effort, these simulators try to cover a wide variety of senses, which can be the visual sense, as the most important cue, as well as a realistic audible stimulation, which optimally includes the realistic simulation of its spatial properties. Additionally, motion platforms can simulate different postures of the subject as well as, to a certain degree, acceleration forces, which stimulates the vestibular senses. Finally, there is a need for implementing force feed back stimuli, for example in the steering wheel or in other devices the subject is interacting with, to address the haptic senses. A mixture of audible and haptic senses can also be stimulated by certain vibrations even in inaudible frequencies to simulate, for example, air streams around the cockpit in a flight simulator. Under such circumstances, as reported by the subjects, a strong impression of reality (immersion) is observable. Therefore such setups are often called high immersive (V.R.) applications.
When it comes to scientific applications, virtual reality provides unbeatable benefits for one special topic, the research of spatial behaviour. When one tries to investigate the movements of navigating humans, many problems have to be solved. There are seldom appropriate environments which fulfil the requirements of scientific work. Then, even if appropriate, they are not always stable, which means that they can differ from subject to subject. Finally, data acquisition becomes difficult in wide range environments. All this problems vanish if virtual reality is used. It provides environments exactly as one likes to create them; they are stable in respect of their physical properties, such as illumination, weather etc., and data acquisition is easily at hand. By using such technology, many aspects of human spatial behaviour have been investigated. Gillner and Mallot (1998) could show that subjects are able to learn routes and the layout of a virtual maze while navigating through it. Wiener and Mallot (2004) investigated the influence of regions which intersect an environment on the route planning strategies. In Maguire et al. (1998), the virtual reality technique is also combined with positron emission tomography. This approach not only allows investigating the behaviour of the subjects while they are navigating, it also gives an insight into the activity of the brain while they are doing so.
Independent from the few people who had access to the high-end applications mentioned above, most experimenters work with V.R. setups which address mainly the visual sense. Although one can achieve feasible results even with such low level devices, the fact that only the visual sense is stimulated by virtual reality can cause some problems. Quite early, when the first military simulators came into use, some subjects suffered from nausea after a certain time in the simulator, this is why this phenomenon was called simulator sickness. It soon turned out that this sickness was caused by a cue conflict between the visual and the vestibular sense. It is therefore closely related to seasickness and other motion sicknesses which some persons feel for example when reading during a car ride. It does not matter if the vestibular sense tells the brain that the body is moving, whereas the eyes do not, like in seasickness, or vice versa, like in a simulator. One explanation for this reaction is the theory that the body interprets the conflicting senses as a sign for intoxication and therefore induces nausea and regurgitation to get rid of the toxic substance. Kolasinski (1995) investigated the causes for the appearance of sickness in a (V.R.) simulator and identified several factors, such as the frame rate of the display and the field of view which was covered.

Recently, virtual reality has also been applied in the therapy of phobias. Botella et al. (1998) gives an overview of some attempts in which a therapy for claustrophobia was performed successfully by using virtual environments. There are also attempts to expand this treatment to several other disorders, such as acrophobia or arachnophobia.

1.7 VR in animals

Since virtual reality proofed to be a suitable tool for investigating the behaviour of humans, there is also a desire to benefit from the advantages of virtual reality for animal experiments. As some techniques, like e.g. electrophysiology during navigation, are not applicable to humans, there is a need to expand this technique on animals.
For insects, virtual reality applications have been constructed which surround the animal with a back projection (Gray et al., 2002) or with an array of LEDs (Dill et al., 1993). These displays are designed to present flow fields to the insect rather than the image of an environment. If a wind source is applied in addition to the flow field, the animals start to make flying movements when they are tethered in the centre of this setup (Fig. 3). Various investigations on the visuo-motor-coupling of flying movements have been performed with such setups.

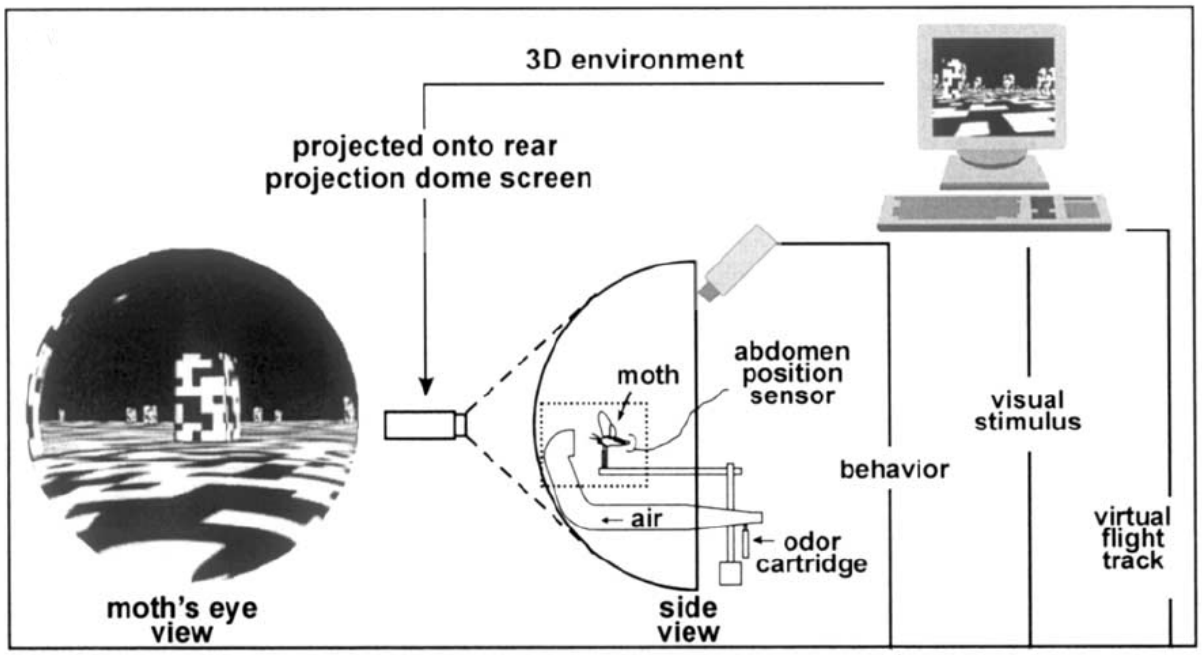


Fig. 3 The virtual reality flight simulator for moths presented by Gray. The output of the rendering computer was sent to an LCD projector which projected the environments onto a rearprojection dome screen which occupied 250° of the moth's field of view. An infrared video camera was placed above the moth. The wind which was delivered through a j-shaped glass tube (1 m/s) caused the moth to make fly movements. From Gray (2002).

Several studies have shown that primates are able to interact with and successfully navigate in virtual environments which are presented to them on a monitor (Leighty and Fragaszy, 2003; Nishijo et al., 2003; Towers et al., 2003). This is most likely due to the fact that the non-human primate visual system is quite similar to the human visual system.

Attempts have been made before to construct a virtual reality setup which can be used to investigate the spatial navigation abilities of rodents by Gaffan and Eacott (Gaffan and Eacott, 1997). They trained rats to discriminate among complex visual scenes which were presented at variable locations in the environment. The animals were trained in a Y-maze made of six monitor screens; the scene in each arm of the Y was defined by the visual display on the two monitors which constituted the walls of that arm. Thus, a given scene could appear in any one of the three arms. However, the experimental results suggested that the animals did not treat the presentation of scenes as a virtual environment in which to navigate, but rather as objects within the real laboratory environment. Our hypothesis why this setup did not turn out to be feasible was that it failed to cover the wide field of view of the rats.
After our setup was already in use and first results had been published (Hölscher et al., 2005), three other research groups reported on setups which enable rats to interact with virtual environments. The first setup, which consists also of a projection system for virtual environments and a walking compensator, is presented in Lee et al. (2007). However, this setup only works linearly and does not allow the animals to rotate and therefore not to orient themselves within the virtual reality. In this publication, only the feasibility of the setup is displayed and the intention to combine it with electrophysiological methods is announced (Fig. 4).

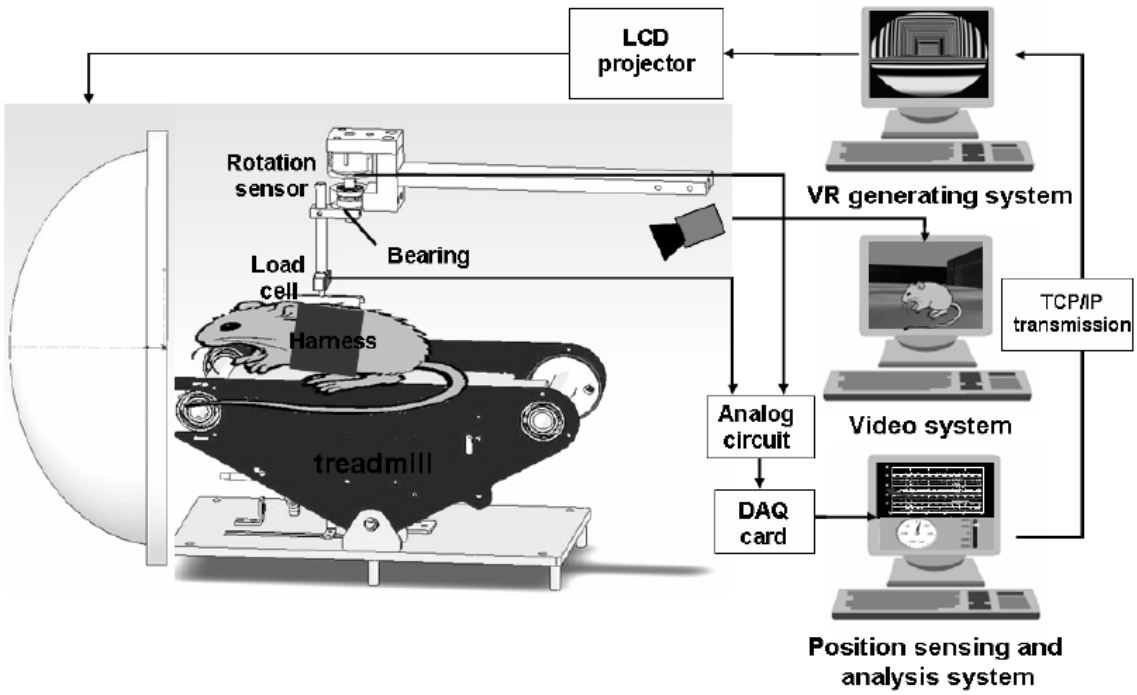


Fig. 4 The virtual reality setup for rats presented by Lee. Again, a back projection screen was used in front of which the animal is placed. The linear treadmill allows the animal to make movements which cause the virtual environment to move accordingly. From Lee (2007).

The second approach is presented in (Dombeck et al., 2007), in which a walking simulator quite similar to the one in our setup is used, although in this case it was not coupled with the projection of a virtual environment. This approach managed to successfully combine the walking compensator with two photon Microscopy techniques and was able to display neural activity in awake, mobile mice (Fig. 5A).
The third and most sophisticated setup is presented by Dr. York Winter from the University of Bielefeld. His setup presents a virtual environment on six computer monitors which surround the animal and therefore cover the visual field for 360°, as in our approach. It also has a spherical walking compensator, but unlike ours, his is actively driven (source: animal-

cognition-systems.com/pdf). Unfortunately, there are no publications available of experiments which have been performed on this setup (Fig. 5B).

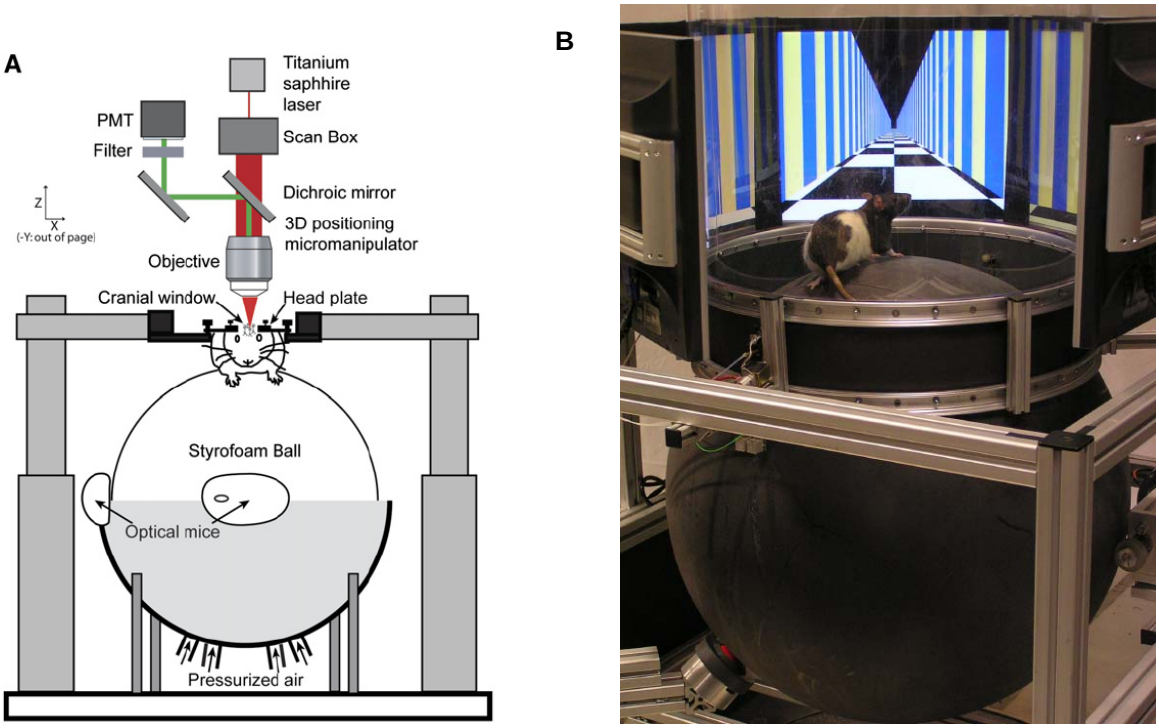


Fig. 5
A: The setup presented by Dombeck lacks a visual feedback. But the free-floating styrofoam ball which acts as a spherical treadmill is quite similar to the one used in our setup. From Dombeck (2007).
B: The virtual reality setup for rats presented by Winter. The spherical treadmill is motor driven and therefore a fixation of the animals is not needed. The virtual environment is presented on 6 or 8 computer monitors, which cover 360° of the horizontal visual field, comparable to our setup. Source: animal-cognition-systems.com/pdf/1.pdf.

2 Material and Methods

The setup consists of two major parts: on the one hand, a running ball as an input device, similar to a joystick, which registers the movements of the animal and, on the other hand, a projection system which presents the view of the computer-generated environment to the animal (Fig.6).

Fig. 6 Overall view of the setup with the screen lowered in working configuration. Left and right of the rack which supports the sphere (not visible), two speakers can be seen.

2.1 The treadmill

The running ball, as a central part of the setup, is in fact just a styrofoam sphere which floats on an air stream. The sphere consists of two hollow, commercially available half spheres connected by normal glue. Out of the factory, these spheres have a thickness of 30 mm, which results in a total weight of 400 g. The weight of the sphere should be approximately 1.5 times the weight of the animal to make sure that the rotational momentum of the sphere requires the animal to transmit the same amount of force to the sphere as the animal would need when moving on solid ground. To adjust the weight of the sphere to the weight of the animals, thin layers of material can be cut out of the inside of the sphere by using a device similar to a lathe with a hot wire, which serves as cutting tool. Following this procedure, spheres with a weight of just 130 g and a thickness of less than 10 mm have been manufactured, which have proven to have still sufficient solidity. In practice, the weight of the animals increased during longer training sessions, and with respect to the big inter-individual variance of weight, we were not able to adjust the spheres' weight exactly to the gram. In fact, a rough mean weight was chosen which corresponded mostly to the original weight of the spheres since the animals soon reached a weight that matched to this. With an average weight of the animals between 200 and 300 g, we did fairly well with the original weight; however, there was no possibility to increase the weight of the spheres without creating an imbalance. To obtain a perfect shape and a certain smoothness of the surface, after cutting and gluing, the spheres were boiled in a custom made aluminum hollow sphere which was manufactured on a CNC (computerized numerical control) lathe and represented a perfect hollow sphere of 500 mm diameter. The

temperature of boiling water (100°) is enough to bring the Styrofoam into a state of plasticity. The gas in the bubbles of the Styrofoam is still under pressure and, when the material is softening, it tries to expand. As a result, the sphere takes the shape of the aluminium mould. Finally, the spheres are painted with a light mist of black dots, which is important for the later prescribed optical tracking. The dots have a size of about 0.1 mm and are generated with a commercially available airbrush gun and black Indian ink.
The final Styrofoam sphere rests in a custom made aluminium half hollow sphere with a diameter of 504 mm on the inside (Fig. 7). This difference in size creates a little gap trough which the air stream can flow which supports the sphere. In the south pole of the aluminium bowl is an opening of 5 cm diameter through which the air is led in. At this inlet, a flow resistance is filling the tube to prevent a free stream in the reverse direction into the supplying tube. When a downward movement of the sphere compresses the air in the tube, the applied pressure causes an overshooting upward movement which leads to oscillatory movements of the sphere. These movements are of course very irritating and must be avoided by all means. The usual working pressure of the setup was adjusted by hand at a pressure regulator and usually came with 1.5 bar.

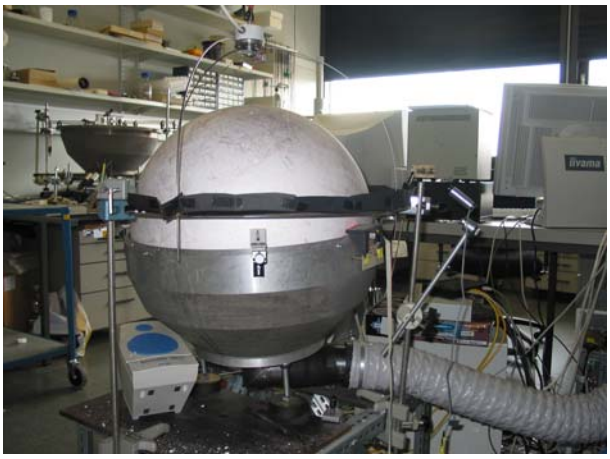


Fig. 7 Detailed view of the sphere in the mould. The compressed air is delivered from below trough the grey tube.

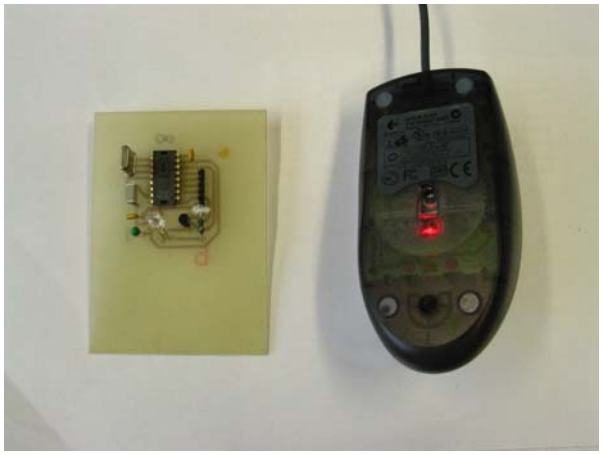


Fig. 8 Detailed view of a sensor plate and a standard optical mouse, where these sensors usually can be found. The correction lens is on the opposite side of the board.

The air stream enables the sphere to rotate almost without friction. In order to make the input device function as we desired, it was necessary to prevent the sphere from rotating about a vertical axis. To achieve this, the aluminium bowl is tilted to the side by 7.5°, which causes the sphere to shift towards this side. On the edge of the bowl, in +/- 45° apart from the tilting direction, two aluminium wheels are mounted which prevent the sphere from touching the inside of the bowl. These two wheels are adjustable in a way that the touching sphere can be centred on the air stream. The sharp edges of the wheels apply enough friction, so that the sphere does not rotate around the vertical axis, whereas the additional friction from the wheels' needle bearing is negligible when the sphere is rotated around the horizontal axes.
In order to make this air floating running ball function as an input device, two motion tracking sensors are required. These sensors are of the type that is used in common commercially available optical computer mice (HDNS2000 Agilent, Palo Alto, CA, USA) (Fig. 8). Apart from a light source which illuminates the detectable surface, the main component of these sensors is a 16 ∗16 pixel camera (size of the light sensitive area = 1 mm x 1 mm), which takes pictures every 0.66 msec and stores them in a buffer. The more important feature of these sensors, however, is their ability to online compute a correlation between two subsequent

images which outputs a motion vector of the shift between the two images in x and y coordinates. The way these sensors are mounted in a computer mouse guarantees that the distance between sensor and surface is constant, which is essential for focusing. Unfortunately, caused by the motions of the sphere, this constant distance can not be guaranteed. With the help of a correction lens (4.6 mm focal distance 1/7 magnification factor) on the camera, the depth of field is enhanced and thus adjusted to the bigger distance and variability. The correction lens enlarges not only the image, but also its speed on the sensor, which leads to a detectable speed of maximally 2 m per second on the surface of the sphere (which equals 30 cm on the light sensitive area). The minimal detectable step resolution is 0.5 mm (which equals 0.062 mm on the light sensitive area). The signals of the sensors are processed by an incremental counter board (APCI1710, ADDI-Data, Ottersweier, Germany) which is plugged in the main control PC.

Two of these sensors are mounted at the edge of the bowl, which corresponds to the equator of the sphere. One of the sensors is aligned with the tilting direction and therefore 7.5° above the equator, the other sensor is placed 90° apart from the first, at the point where the edge of the bowl crosses the equator. In this constellation, the y components of the two motion vectors together are sufficient to describe the x and y motions of the sphere (Fig. 9). However, to control for rotations of the sphere, the x components of the sensors' motion vectors are also registered.

Fig. 9 Detailed view of one of the two sensors at the X position. The black cardboard provides a light shield which protects the sensor from interfering light sources.

2.2 The rat fixation

The whole apparatus with bowl and sphere is mounted on a rack which also holds the fixation mechanism for the animals. Ascending from a steel ring which surrounds the bowl on equator height, three steel wires 120° apart from each other and with a diameter of 3 mm follow the shape of the sphere in a distance of about 5 cm and meet in the zenith of the sphere. In the meeting point of these three wires, an angular incremental counter (Typ RI 58-D /1024, Hengstler, Aldingen, Germany) is located which serves as a rotational joint for rotations around the vertical axis. Beside the position data from the motion sensors, this sensor provides additional information on the body orientation during the experiment.

On the lower side of the sensor, two magnets are attached to its rotation axis. These magnets serve as quick lock connection between the setup and the animals (Fig. 10). From the lock, a thin aluminium stripe is projecting to the animal; hinges at both its ends allow vertical movement of 60 mm at the free end. To finally fix this hinge joint to the animal, the latter have to wear a leather jacket. Two stripes of Velcro ® fastener on the sides of the jacket allow the connection with the hinge joint.

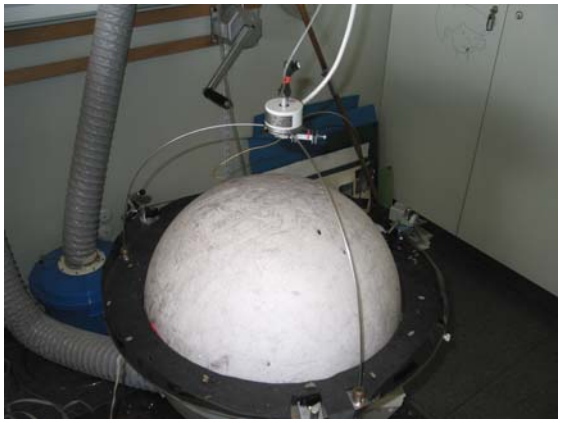


Fig. 10 Top view of the sphere and the surrounding wires which support the rotational joint. Below the white angular counter case, the magnets of the quick lock can be seen.

Fig. 11 Image of an example jacket and of the hinge joint which connects the jacket to the rotational joint.

The jackets are custom made out of suede and have two holes trough which the front legs are projecting. On the back side, the jacket is closed by four stripes of Velcro ® fastener which allowed us to adjust the size individually. According to the size and weight of the animals, which varies beyond the range of the fastener, jackets in two different sizes have been manufactured to ensure a secured fit. The shape of the jackets is chosen in a way not to impede the free moving space of the animals too much, however, to ensure that the animals are not able to slip out of the jacket too easily, a compromise between both wants was needed (Fig. 11). The natural behaviour of a rat includes a lot of postures in which the animal sits on its haunches and hind paws, which allows it to lift the fore paws of the ground, for example during food uptake or during grooming. These postures go along with a curved body or, to put it simple, the rat makes a hunchback. As mentioned above, the jackets we are using are closed on the backside, which makes them rather stiff on this side and does not allow the animals to adopt this ducked position. In a usual walking position, all four paws of the animal touch the ground and the back is more or less straightened, for these postures enough free moving space is provided. The locomotive behaviour of a rat changes from walking to trotting and finally galloping with increasing velocity. When walking on the sphere, normal walking behaviour can be observed after a certain time of familiarization with the setup. Sophisticated rats show also trotting behaviour. Galloping behaviour is usually not observed, it only occurred when the animals were startled by an accidental event. Such events need to be prevented by all means because under those conditions, the free moving space of the fixation system is at its limits and an accidental release can occur (Fig. 12).

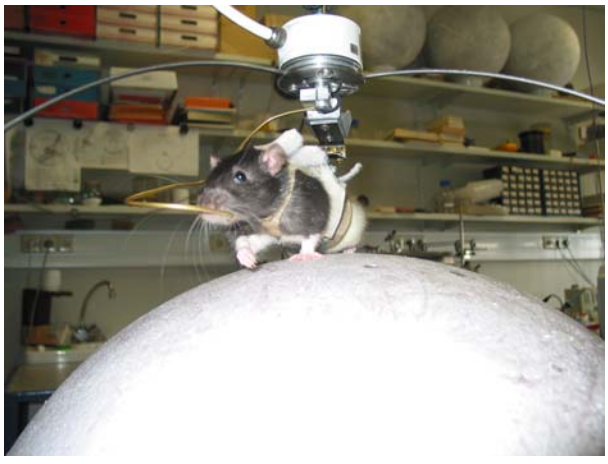


Fig. 12 Image of an example animal, which is fixed on top of the sphere by the jacket and the hinge joint. The brass tube of the reward system can also be seen in its working position.

2.3 The projection

The output part of the setup is a projection system which illuminates a 360° screen.
The 360° projection is achieved by using only one standard video projector and a special mirror system. In the zenith, 1.2 m above the animal and with a tilt of 30° downwards, the video projector is mounted on a support system which is suspended from the ceiling. The projector is a commercially available DLP (digital light processing) projector (Liesegang ddv 1800, Liesegang, Düsseldorf), which runs with a resolution of 1024 by 768 pixels at a frame rate of 66 Hz and 1800 ANSI-Lumen (Fig. 13A).

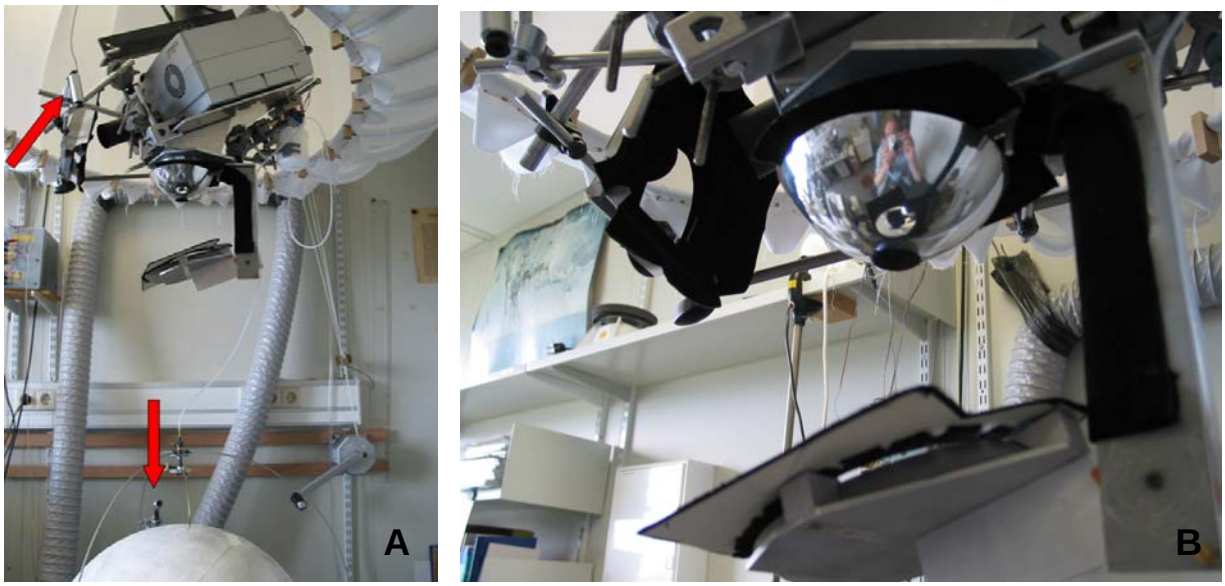


Fig. 13
A: The projector in its position within the setup, with the screen in maximal upper position. The position of the two observation cameras is highlighted by the red arrows.
B: The mirror system with the two plane mirrors and the spherical mirror.

To meet the requests of our mirror system, the optic of the projector has been modified. Unlike most available projectors, which come with a wide angel lens to achieve a maximum image diagonal at a minimum distance, our projector is equipped with a telephoto lens of a focal length of 90 mm. This lens creates a mage of 120 mm diagonal at a distance of 420 mm, which is measurable at the second mirror of the mirror system (see below). The projector does not only request a rather small image diagonal, but also needs to be focused on a fairly short distance (approx. 570 mm). Commercially available projectors are usually not designed to cover such small distances, especially not the ones with a telephoto lens. Although it was not designed that way by the producers, our type of projector allows shifting the complete lens apparatus within the focusing device. Due to these modifications, the projector is able to create well focused images of a small diagonal at short distances. The crucial part of the projection system which generates these special requests to the projector is the mirror system (Fig 13B). It consists of two plane and a convex mirror. The two plane mirrors at a distance of 120 mm and 420 mm serve as tilted mirrors. The third, convex mirror is fixed underneath the projector with its curved reflecting side facing downwards and is centred in the zenith of the running sphere. This convex mirror is a polished rotational symmetric aluminium surface with a vertical rotation axis, which widens the elevation of the incoming light bundle by a constant factor of 39° over its whole surface. The outer edge of the mirror has a radius of 60 mm. This is the reason why the projector needs such special properties. The image needs to be focused on the mirror and, to avoid a loss of to many pixels, it should not be larger than the mirror diameter. The beam which is reflected from the mirrors illuminates the projection screen

surrounding the mirror (red beam in Fig. 14). This screen has the shape of a torus with a horizontal diameter of 1400 mm and a ring radius of 400 mm. Along the rotation axis, the torus is hollowed out and leaves an opening of 720 mm on both sides. This toroidal shape consists of 24 segments of white elastic cotton, which is clamped into the desired shape with the help of a wire frame that contains 24 segments, too. The complete screen is suspended from the ceiling at thin nylon ropes which end up in a hoist that allows varying the elevation of the screen. The screen is in the right position for the projection when the lower edge is at the height of the equator of the sphere. In this position, it completely surrounds the running sphere. Therefore the lifting mechanism is essential if one wants to gain access to the fixation device of the running sphere to attach or detach an animal. From the lowest, respectively the projection position, the screen can be lifted up to 800 mm. The screen acts as a horopter. If prolonged, the sectional shape of the torus forms a circle. Since the position of the mirror and the position of the animal are points of this circle, every point on a vertical axis on the screen is reflected under the same angle from the mirror to the animal (blue beam in Fig. 14). The complete projection system ends up in a vertical field of view from -20° to +60° and a horizontal field of view of 360° with respect to the position of the animal (green area in Fig. 14).

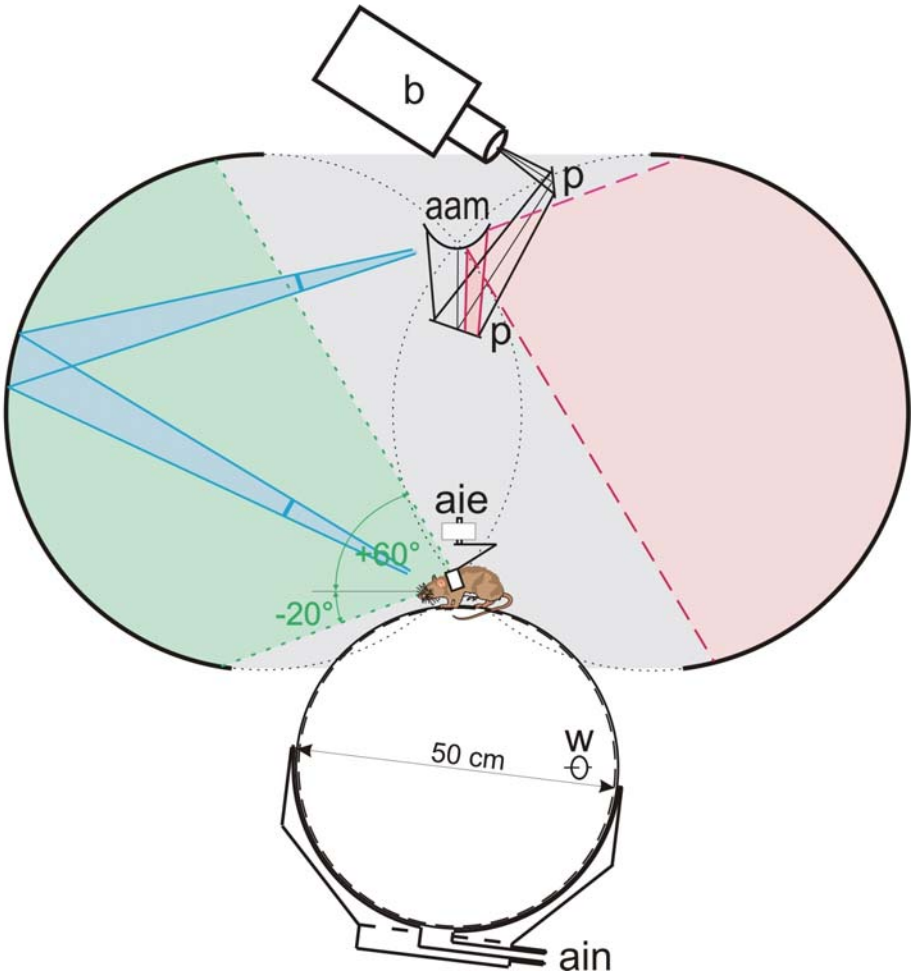


Fig. 14 Schematic cross section of the setup. The red area represents the beam from the projector. The green area represents how much of the rats visual field is covered by the projection. (b-beamer, p- plane mirrors, aam- angular amplification mirror, aie- angular incremental counter, ain- air inlet, w- support wheel).

2.4 Additional elements of the setup

Beside the core components which provide a closed system of input and output devices the animal is interacting with, there are some further important devices which support the applicability of the device. The main feature of a setup designed for behavioural experiments on animals is the possibility to apply reward. With respect to the fact that the animal can rotate freely in its fixation and to other technical problems, it is easier to apply liquid reward than solid pellets. Through the vertical rotational axis of the angular incremental counter, a brass tube of the diameter of 3 mm is led which ends directly in front of the animal's snout (Fig. 12). Since this tube is fixed to the rotation axis, it stays in place regardless of the movements of the animal. The tube ends 20 mm above the angular incremental counter and is then connected with silicone tubing to the depot for the liquid. The elasticity of the silicone tubing is sufficiently strong to withstand multiple revolutions without collapsing, which made a swivel connection dispensable. The depot is a glass bottle with a volume of 50 ml, which is fixed beneath the projector. The height of approximately 50 cm above the outlet is sufficient to ensure a reliable flow trough the thin tube. Right beneath the depot bottle, an electromagnetically activated squeeze valve is attached to the tube. In all of the following experiments, an opening time of 100 msec was used to deliver a rewarding liquid drop. The liquid was a solution of 50 g sugar (saccharose) in 50 ml of tab water. This high concentration of sugar resulted in a liquid with high viscosity, which was desired to ensure that the delivered drop sticks to the tube. Liquids of a lower viscosity would have dropped instantly onto the sphere, which causes a rapid soiling of the sphere and additionally a loss of the reward. Between the experiments, the complete depot bottle containing the sugar solution was kept in the fridge and after two or three days it was renewed. After each experiment, the rewarding apparatus was rinsed repeatedly with tab water to prevent sugar incrustations which otherwise would have occluded the tubes.
In most of the later described experiments, only the reward system was used, but more recently an additional punishing device was installed which is closely related to the reward device. A second brass tube, exactly like the tube of the reward device, was installed through the axis of the angular incremental counter. On the end of this brass tube, which terminates at the same position as the former, a silicone tube was attached, as well. Another electromagnetically activated squeeze valve at closely the same position as the first one controlled the stream through the tube. But in contrast to the reward device, the end of the tube had a direct connection to the air supply driving the running sphere. A second pressure regulator allows adjusting the pressure independent from the pressure which is delivered to the running sphere. With a pressure of 1.5 bar, the opening time of the valve was set to 200 msec to produce a rather strong air puff. The strength of the puff was chosen intentionally to avoid habituation to it. Although such a puff can startle the animals and seems to be fairly uncomfortable, it does not harm them, like for example electro shocks would do. This is essential because even after receiving an air puff, the animals should still be motivated to continue their explorative behaviour, while an electro shock can cause noting but freezing behaviour.
With the screen lowered to projection position, the animals cannot be seen from the outside. To constantly observe the animals during an experiment, two cameras are installed at the setup. One of them is attached to the metal frame which surrounds the equator of the running sphere and delivers side views. The second camera is fixated at the front of the projection system and delivers top views (Fig. 13A). The images of these cameras are usually not recorded since this information was not used for behavioural analysis. The main purpose of these images is to control for unforeseen events, such as, for example, an accidental release of the fixation system.

During an experimental session, the special events like a reward and later the punishment were mostly accompanied by a simultaneously played sound file. The sound output was accomplished by two commercially available desktop stereo speakers which were placed to the lower left and lower right side of the running sphere (Fig. 6).
The final instance of the setup, where the output and the input information's of the device converged, is the control PC. This standard PC is equipped with a 1200 MHz AMD Athlon processor, 500 Mb RAM and a NVIDIA Gforce 3 graphics card with 64 Mb RAM. Sound is generated by a 128 bit sound card. The only unusual part of the PC is the incremental counter board mentioned above to which the signals of the motion sensors and of the angular incremental counter are fed. The card also generates signals which operate the valves of the rewarding and the punishing system. An additional signal from the card can also be used to synchronize a second computer with the control PC. Since the graphics card of the control is busy with generating the images for the projection, a second PC was used to display the images of the two observation cameras.

2.5 The software

To display a 3D object in a computer, a so-called model of the object needs to be created. Basically, such a model contains just a list of X/Y/Z coordinates which define points in the space (vertices). Always three of those vertices are connected by edges to form a face, a multiple of which can be combined to a so-called polygon. To create, for example, a cube, one defines eight vertices which are connected by 18 edges to form 12 faces, two of which always form one of the six planes (polygons) of the cube. To make this process easier and also applicable for complex objects, a programme is available which supports this procedure with several tools and a GUI (graphical user interface). All of the following environments are created with the help of the modelling software MulitGen (Firma MultiGen Paradigm Inc., Version 2.5.1).
The control PC was running with the operating system SUSE LINUX 7.1 (later upgraded to version 9.3). To display interactive 3D environments, the graphic engine Open GL Performer (SGI, Mountain View, CA, USA) was used.
The main programme which runs the experiments, controls the setup (magnetic valves, optical sensors, sounds) and operates the graphic engine was programmed in C++.

2.6 The animals

All animals were male animals of the Long Evans breed, which were purchased at the age of approximately 4-6 weeks at Charles River laboratories. In our laboratory, groups of two were kept together in one standard housing box, maximally 8 of which were stored in an air-conditioned cabinet (scantainer, Scanbur A/S, Denmark). At the above mentioned age, the animals were slightly too small, respectively too light to fixate them on the V.R.setup. For this reason, the animals received food at will (ad. lib.) until they reached a minimum weight of 200 g. During the experiments, the animals were fed following a diet of 15 g of standard rodent food pellets. This diet was rich enough to allow the animals an additional weight gain of two to three grams per week. The dietary fare was also the reason why just two of the animals were kept together in one cage. More than two animals per cage could easily have led

to a situation in which a dominant one gets too much or a subordinate animal receives an insufficient amount of food. The two-animals-per-cage housing was a compromise between the dietary situation and a prevention of total social isolation. But also with two animals per cage, such rivalry can occur; the weight of the animals was therefore monitored every second day and, when needed, a regrouping or an isolation took place. When isolation was inevitable, it was tried to keep the isolation time as short as possible.

2.7 The handling procedure

During the time of weight gain, the first step of the handling procedure was performed, i.e. the experimenter started to get the animals used to human contact by petting them. On the third day, they first experienced the procedure of wearing the jacket for a short amount of time. This was repeated on the following days, eventually several times during a handling session in the latter days. At this time the animals also came into contact with the rewarding sugar solution. This happened, on the one hand, by administering a small amount of the sugar solution trough a small water bottle directly in to the cage for three or four days, depending on how quick the animals accepted and drank it. On the other hand, they received the sugar solution through a modified syringe, which had, as a tip, the same type of brass tube as the rewarding device in the V.R. setup. With the help of this syringe, the animals were hand fed while they had to wear the leather jacket. This procedure was thought to associate the "uncomfortable" jacket with the reward. In the beginning, the animals refused to accept the presented sugar solution, but after approximately one week all animals were well habituated to the whole situation and relaxed enough to accept the procedure. The above mentioned time of weight gain lasted on average two weeks. After several handling procedures, it turned out that the moment when the feeding changes from ad.lib. to dietary (15 g per day) is the ideal time to get them used to the reward mechanism in the V.R. setup. During the second week of the handling procedure, the animals were transferred to the V.R. setup room, which allowed them to get used to the transport procedure and to get accustomed to the soundscape of the V.R. setup room. On the last day of the weight gain period, the usual jacket wearing and handling procedure was combined with placing the animals on the running ball, i.e. fixating them in the setup, however, still without starting the projection. In this first encounter with the setup, the animals were usually very nervous and frightened, so that it almost never occurred that they accepted sugar solution from the rewarding tube. To keep this agitating episode short, the time on the ball was limited to one minute. When the handling procedure was accompanied with a mild food deprivation due to the change to dietary fare on the second day, it turned out that most of the animals accepted the sugar reward already by then. To fully associate the reward with the setup and the respective situation, this procedure was repeated for another four or five days. This resulted, depending on the duration of the weight gain period, in a total handling time of approximately two and a half to three weeks.

3 Experiments

3.1 Preface

The following experiments section contains a total amount of 18 experiments which were performed with the rat V.R. setup system. Although many of these experiments did not show the desired outcome or revealed an unexpected behaviour of the tested animals instead, I decided to present all of them here to give a complete overview of the process we underwent to create and adapt the experimental procedures, the environments and the controlling programs in order to reveal our V.R. setup as a practical tool for investigating rat behaviour. Although some of the experiments turned out to have a greater importance than others, I will stick to the timeline in which these experiments were performed in order to outline the learning process which allowed us to improve the experimental setup from one experiment to the next.
The changes between the experiments regard mainly alterations of the virtual environment and of the programmed routine which controlled the course of events during the experiments. For this reason, the material and methods sections of the following experiment descriptions concentrate mainly on these changes. When there were changes in the setup, as it was described in the previous chapter, I will refer to them at the respective point.

3.2 Experiment 1

3.2.1 Preface to Experiment 1

During the construction of the setup by Dr. Hansjürgen Dahmen, some pre-tests of the setup were performed, which led to the first major constructional changes. The first design of the setup did not allow the animals to rotate around their vertical axis, meaning that they had to perform turns by rotating the ball, which was possible due to the absence of the equatorial aluminium wheels which later prevented this rotation. In this configuration, the animals were not able to walk straight ahead since rotational momentums of the ball summed up and forced the movements of the animal onto a spiral-shaped trajectory. Although these pre-tests did not last very long, they left some imprints on the animals. With this first set of animals, Experiments 1-7 were performed. It could be observed that, at least at the beginning of the first experiments, the animals tended to run on a spiral-shaped trajectory or were at least influenced by one.

3.2.2 Introduction

The first experiment was designed with the intention to reveal the feasibility of the setup. The main questions hereby were, firstly, if the animals could interact with the setup in an

appropriate way and, secondly, if they reacted to any object which was presented to them via the projection system. To answer these questions, we introduced the suspended columns described below.

3.2.3 Material and methods

The virtual environment in this experiment consisted of a squared patch of ground floor of 2 m * 2 m size. 1 m above this ground plane, another patch of the same size was attached which served as ceiling. From this ceiling, four columns were suspended in a way that they did not reach the ground. The columns measured 30 cm in diameter and ended 30 cm above the ground, which gave them an overall length of 70 cm. The columns were positioned with their centre 50 cm in x and 50 cm in y direction from the outer edge of the ground patch, so that they formed an equally distanced grid of 1 m * 1 m. The ground patch was textured with a grass-like image, whereas the ceiling had a brick wall texture. The columns themselves were untextured, but were coloured each in a different pastel tone of the colours green, red, yellow and blue (Fig. 15). 49 of these patches were arranged in a square with a size of 3 patches on each side (Fig. 16).

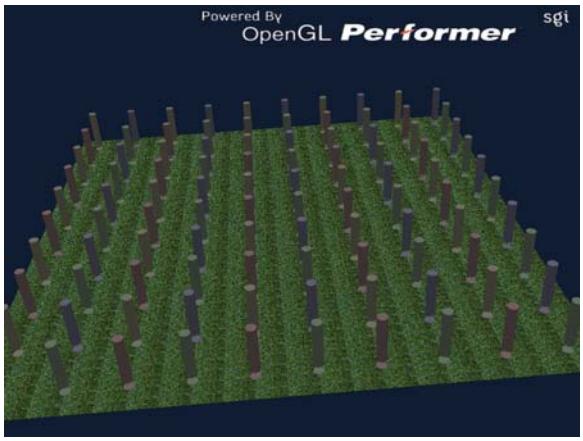


Fig. 15 Inside view of the environment in Experiment 1; the actual view height of the animals during the experiment was lower.

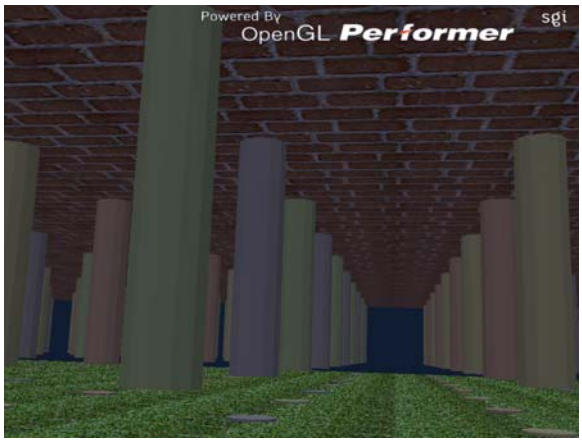


Fig. 16 Top view screenshot of the environment in Experiment 1.

Seen from the centre patch, the animal was surrounded in every direction by three patches or respectively eight rows of columns. The program for movement control was, in this experiment, set in a mode we called repetitive, which means that whenever the animal crossed the border of the centre patch, the complete environment was shifted to the opposite edge of the patch. Due to the fact that this shift took place within one frame (ca.1/100 sec) and to the overall repetitive structure of the environment, this shift was not noticeable and resulted in a seamless motion trough an infinite environment. The only change, which, however, could hardly be observed, was the appearance of a new row of columns at the far end of the horizon. As an experimental condition, the animals had to learn to run underneath the columns. Therefore, the program gave a reward every time the animal entered the 30 cm diameter sized area in the relative position to the column on the ground. The reward was a drop of sugar water which was delivered as described in Chapter 2.4. The rewarding event was also marked by the sounding of a “ding”-called wav file (one of the standard implemented windows system sound files). For the experimental procedure, it was desired that the animals run from one column to the next one; therefore we implemented the condition in the program that, after receiving a reward at one column, the animals had to move away at least 1 m before they

could receive a second reward at the same position. The experiment ended by decision of the experimenter.
The animals were six male Long Evans rats which had previously been used for several experiments by Dr. Hansjürgen Dahmen, as mentioned in the preface of this chapter.
The experiment was performed on eight consecutive days.

3.2.4 Results

In comparison to the previous test trials, we could observe that the rats' movement and the interaction with the setup improved dramatically. Having the freedom to rotate on top of the ball, the animals were able to chose a certain direction and follow that path on a straight line.
Fig. 17 displays three example trajectories which show the several stages the animals went through. Fig. 17A is an example from the first day of the experiment, in which we can see that the tendency to walk in spirals is still strong and is not helping to receive a reward at all. The trajectory in Fig. 17B shows an intermediate stage with a mixed behaviour. In the beginning, the animal still shows the spiralling behaviour, whereas after a certain time, it decides to orient itself at the virtual environment and straightens its path by running towards the columns. In Fig. 17C, a trajectory of the later days of the experiment is displayed in which almost no spiralling behaviour was observable.

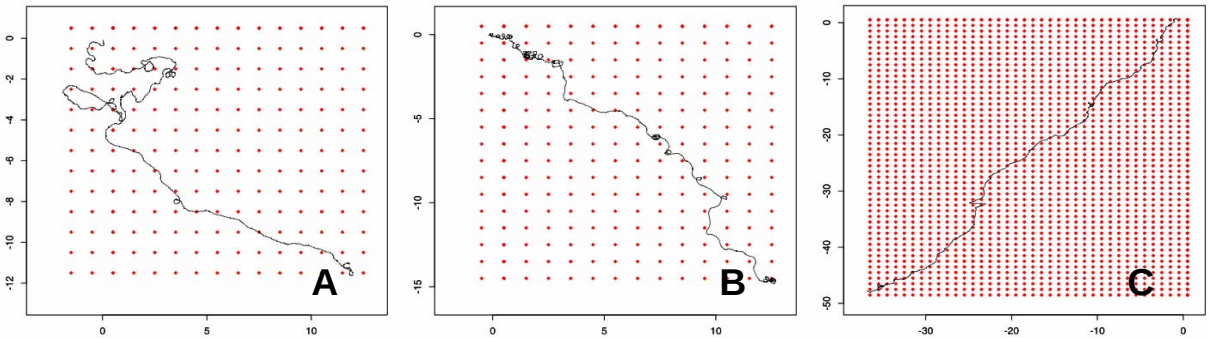


Fig. 17 Display of three example trajectories from the first, the third and the last day of the experiment. The size of the red dots marks the positions of the columns, but does not reflect their diameter.

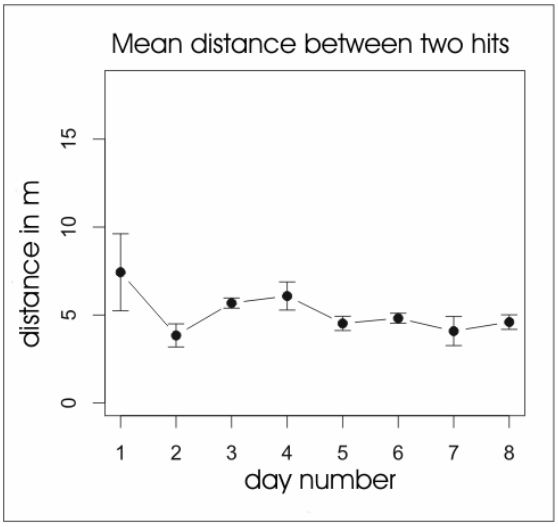


Fig. 18 Display of the mean distance between two hits over all eight days.

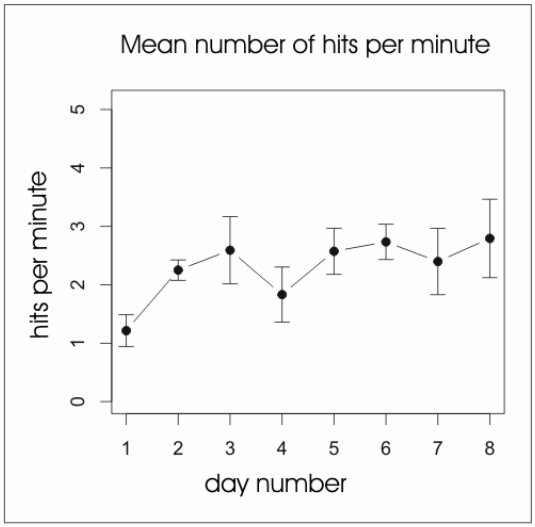


Fig. 19 Display of the mean time interval between two hits over all eight days.

Although the spiralling disappeared in the course of time, the number of column hits was not very convincing. A learning phase between day 1 and day 2 is observable in Fig. 18, but after these days the performance reaches a plateau. The final value for the mean distance between two hits is around 5 m and far away from the ideal distance of 1 m. The same development of the performance can be seen in Fig. 19, where the mean time interval between two hits is displayed.

3.2.5 Discussion

Although the performance values of the given task were not convincing, the experiment itself was rated as a success because the animals had shown that they could move quite naturally on the ball and, even though they faced such an unnatural setup, it appeared to us that after a certain habituation period they enjoyed to interact with the setup as they do enjoy moving on an ordinary running wheel for rodents. The question why the performance level remained so low, however, is still open. Subjective observations of the animal's behaviour convinced us that the animals tried to move towards the targets, but often failed in applying the necessary fine motor skills to the ball to do so. Another explanation could be that, due to the colouring of the columns, brightness and contrast levels against the background were not big enough to be discriminated. The observations we made during the experiments, e.g. when we saw an animal turn around after hardly missing a column to try again, convinced us very much that we were dealing with a real interaction with the virtual environment, only the data we had by then were not convincing enough.

3.3 Experiment 2

3.3.1 Introduction

The question if the animals were able to recognize elements of the virtual environment as objects was at this time still open. Thus, we tried to implement a standard task of behavioural research, the discrimination task, which has been used for a lot of psychophysical investigations. We hypothesized that if the animals could discriminate between two visually different objects in a virtual environment, they had to recognise them as objects, which we hoped to identify by their moving behaviour. The doubts we had concerning the colouring rising from the first experiments also found entrance in the design of the environment for Experiment 2.

3.3.2 Material and methods

The environment in this experiment was the same repetitive setup as it was described for Experiment 1 in Chapter 3.2. One difference to the former environment was that the repeated central patch had a size of 4 m $*$ 4 m. There also were four equally distanced columns distributed on this central patch, which resulted in a column to column distance of 2 m. The ground patch was again textured with a grass image, whereas the ceiling received a "cloudy blue sky" texture this time. Finally, the main difference lay in the suspended columns, of

which there were two different ones. In order to make them highly distinguishable, they differed not only in colour, but also in shape. One of the columns had the shape of a double truncated cone connected at the broad base and touching the ceiling with its small ending. This cone bore horizontal, black and white stripes. The other column had the shape of a truncated cone with the broader base towards the ceiling. In the first half of the experiment, it was of dark grey colour, whereas in the second half, it was vertically black and white striped. The respective thin ends of both cones, which pointed towards the ground, had a diameter of 60 cm at the tip, which was enlarged with respect to the doubled distance between the columns. For a screenshot of the environment see (Fig. 20).

The part which made this a discrimination task was the fact that only one type of the columns was rewarded. The procedure itself was the same as in the previous experiment. The tested animals were also the same as before. This experiment was performed over 18 trials, two of which took place on one day, one in a morning and one in an afternoon session. The experiment can be split into three parts, in which we adapted the environment because of the lack of expected results. During Part 1, which lasted 4 trials, respectively two days, the striped cones were rewarded, whereas the grey double cones were unrewarded. For Part 2, which lasted eight trials or four days, the grey double cones were rewarded and the striped cones were unrewarded. For the final third part, the grey double cones became also striped, but horizontally, in contrast to the vertical stripes of the single cone. In this part, the horizontally striped double cones were the rewarded ones.

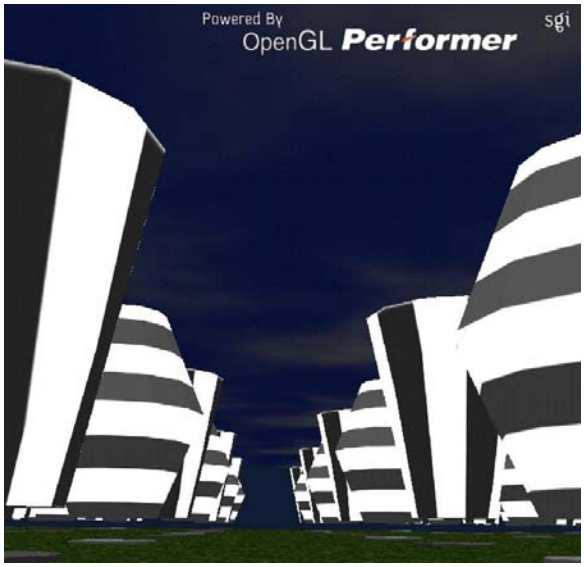
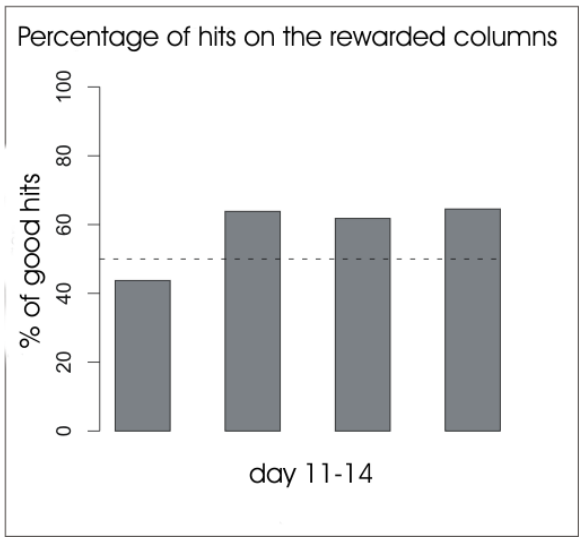


Fig. 20 Inside view of the environment in Experiment 2; this image approximately renders the view height of the animals during the experiment.

3.3.3 Results

When discriminating between two alternatives, the chance level for doing it right is 50 %. The results which we received in the final days of the experiment showed us that the animals' performance differed from this chance level only by +/- 5 %. Neither could we see a performance change over time or between the three different sub conditions. In Fig. 21A, the error rates for the last part of the experiment are displayed. The values for the hits per meter, which are displayed in Fig. 21B, combine the data of Part 2 and Part 3. For this plot, hits of any column, whether rewarded or not, were counted. Although a slight improvement over the 12 trials is visible from 6.8 m to 4.6 m, the final value is more than twice as high as the ideal value of 2 m. However, compared to the values of the previous experiment, a strong improvement in column hits can be observed, which was a result of the additional training phase and the double size of the targets.



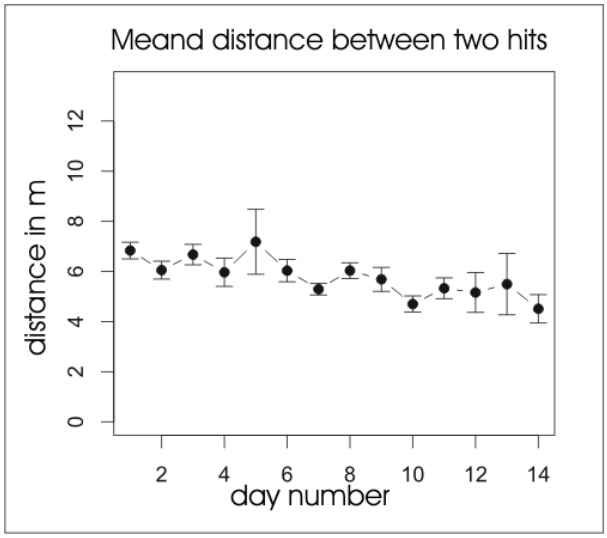


Fig. 21
A: The error rates for the last four days (11-14) of the experiment are plotted against chance level (dashed line).
B: Displays the values for the hits per meter for both parts of the experiment.

3.3.4 Discussion

During this experiment, we were confronted with a problem which would occur on multiple occasions in later experiments. Although we first thought that the discrimination did not take place because of the colour or the pattern of the columns, we later became aware of the fact that the arrangement of the targets on a squared grid was fatal. On the squared grid, every rewarded column is surrounded by four unrewarded columns. To get from one rewarded column to the next, the animals had to choose the diagonal way and neglect the closer one. As we could observe, the animals tended to follow the grid in a rectangular fashion, as the example trajectory in Fig. 22 demonstrates. What now became apparent was the effect that the animals simply enjoyed to run on the ball and therefore did not try to minimize the distance between rewards. The small difference between the diagonal distance (2.8 m) and the double column distance (4 m) is not significant enough to force the animals towards a certain movement strategy. In other words, if the running itself is fun enough, it does not matter if only every second target is rewarded. As we will later see, this is a problem which influenced many of the following experiments.

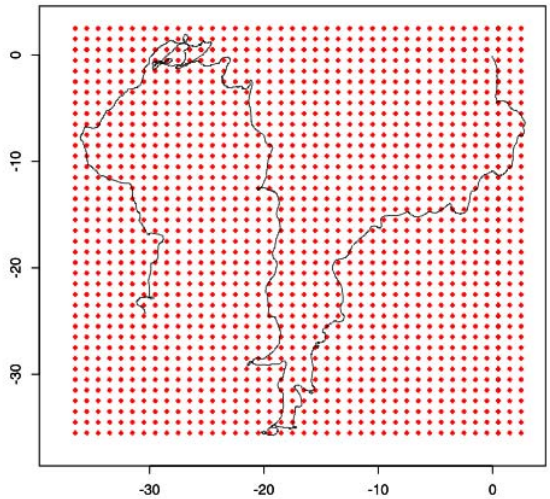


Fig. 22 Display of an example trajectory from the last day of the experiment. The size of the red dots marks the positions of the columns, but does not reflect their diameter.

3.4 Experiments 3,4,5,6

3.4.1 Preface

These experiments represent a certain trial and error phase in our experimental history.
Within a relatively short time span, we introduced many decisive changes in the experimental procedure. With every major change we introduced, we used a new experimental number, which, in retrospect, was unnecessary. Therefore I decided to summarize these experiments in one chapter.

3.4.2 Introduction

The central question concerning the recognition of objects was still open after Experiment 2 and, having the failure of the environmental design for this experiment in mind, we decided to simplify the new environment and the task within it as much as we could.

3.4.3 Material and methods

The new environment consisted of nothing else than a ground and a roof plate, which were again 1 m apart from each other. Those plates were round, had a diameter of 50 m and displayed a checker board texture. In the centre of these plates, one black and white striped column of 50 cm diameter was suspended from the ceiling. The animals started at four different starting points, which were located at the corner points of a square with 8 m side length and the target column in its centre (Fig. 23). The respective starting point was chosen by the experimenter by pressing one of four buttons on the computer keyboard. This led to an immediate shift of the complete environment towards the new position.

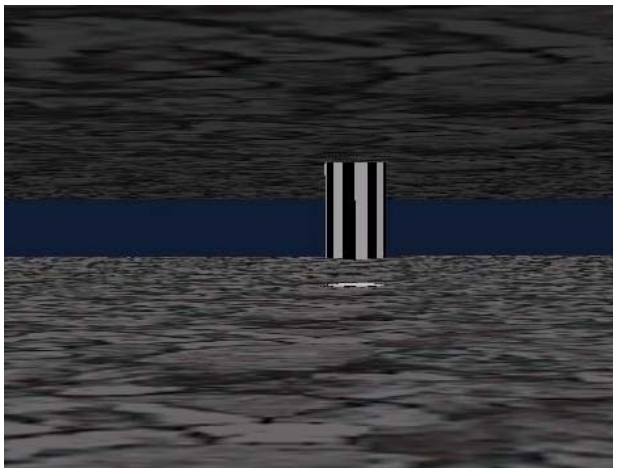


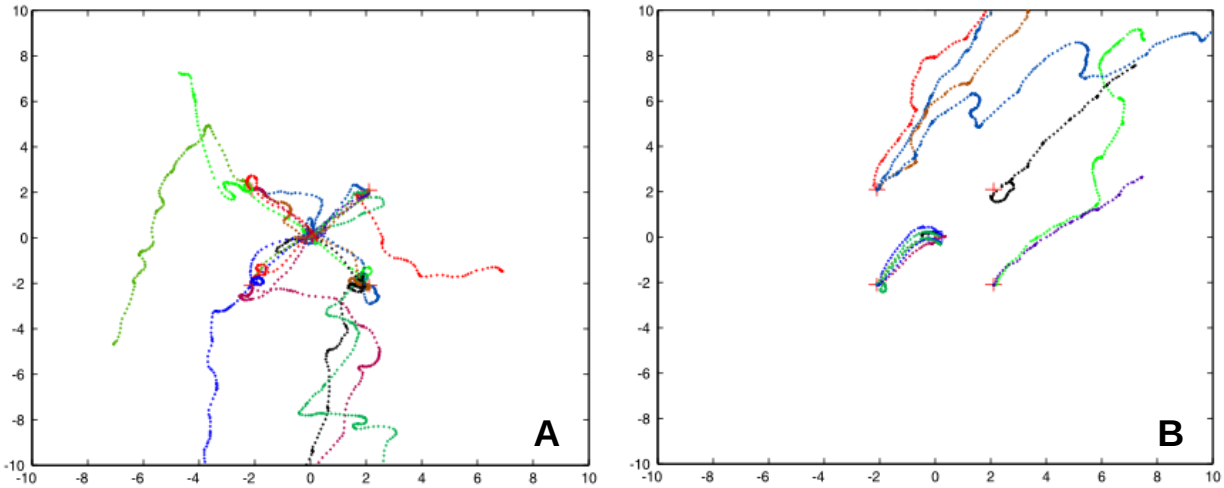
Fig. 23 Inside view of the environment in Experiment 3-6 with the chequer board pattern replaced by the gravel texture; this image approximately renders the view height of the animals during the experiment.

The first change which was applied was a change in texture. The chequerboard texture influenced the animals within a short time to run along the grid or diagonally to it. Therefore the texture was exchanged by an irregular gravel pattern, which was coloured fairly dark to provide a strong contrast with the target column.

The second change was initiated by the observation that the animals did not turn around when they started into the wrong direction. To keep the animals from running all the way to the edge of the ground plates, we defined a maximum distance of 15 m from the centre of the environment, respectively the target. When the rats exceeded this distance, a shift towards a random start place was initiated.
A third change was introduced when we recognised that the immediate shift towards a starting point was not necessarily recognizable, depending on the actual gaze direction. To mark these events clearly, we played another sound, which was again a standard M.S. Windows alarm signal, the so-called "ring" file. Additionally, the complete environment became bright white for a second.

3.4.4 Discussion

The disturbing outcome of this experiment was not presentable as data, but at least it showed us that the design of the environment and the experimental course can implement a lot of misleading errors. One influence which made us carry out the first change in the setup was the influence of the floor and the ceiling pattern. In an environment like ours, with no other cues than the single column, these patterns have a quick and intense influence on the moving behaviour of the animals. Another factor which emerged during this experiment was an enormous diversity between the animals. While animal 204 showed a good ability to run towards the target from various directions in the later trials, animals 202 and 199 completely refused to cooperate and explored the environment for only a few meters. This is also connected to the following strong influence which appeared during the course of the experiments. After we had surveyed the trajectories, it became evident that many animals preferred to run in a fixed direction. We concluded from this that directions might be influenced by fix cues from outside the setup or by the setup itself. The big inter-animal diversity might be explained by their susceptibility to these influences.
The best performance can be seen in animal 204 (Fig. 24A). Although it sometimes ran into the periphery, it performed several successful approaches to the target from all four directions. Then there were animals which followed a fixed running direction, which caused them to run towards the target only if its orientation was more or less towards their fixed direction (see Fig. 24B). Animals which pursued this strategy very strictly and followed a fixed direction parallel to the sides of the square never hit the target, as one can see in Fig. 24C. This finally leads us to the deniers, which became frustrated when not receiving enough reward.



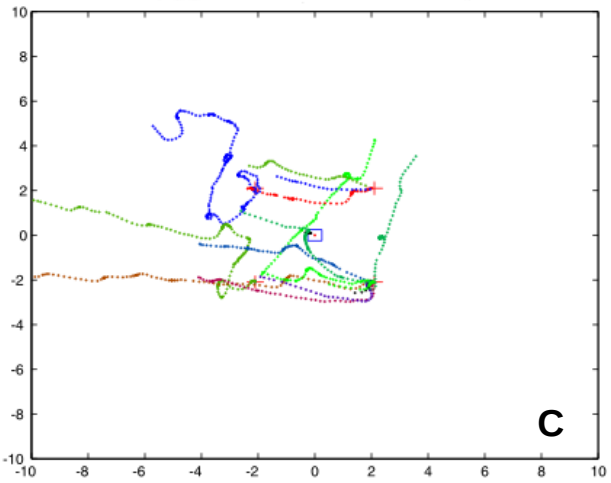


Fig. 24 Display of three example trajectories from experiment 6 (A: animal 204, B: animal 201, C: animal 203). The colour of the trajectory changes after every shift event.

Another element in this experiment which aroused many discussions was the shift towards a random start place. It was clear to us that such instantaneous switches of the complete environment were fairly unnatural, but, on the other hand, our complete setup was fairly unnatural and so we decided to implement them. Due to the lack of any force feedback which we could apply, these transfers were the only possibility for us to guide the animals into a certain direction.

3.5 Experiment 7

3.5.1 Introduction

A prominent feature of the visual perception is detection of optic flow, and though it is well known that it plays a major role in insect navigation (Lehrer et al., 1988), its influence on mammal behaviour is fairly unknown, aside from some investigations on humans in virtual environments. One advantage of using the virtual reality technique is the possibility to decouple the body motion from the visual motion or, in other words, the optic flow. To investigate the influence of optic flow, we tried to start with just one feature, its rotational component. We intended to confront our animals with a rotating environment comparable to those in experiments which were performed on fruit flies (Heisenberg and Wolf, 1993).
In this investigation, a clear oculomotoric coupling between a visual input and a motoric response could be measured when the investigators simulated a rotation of the environment. The following experiment should clarify if rotational optic flow can also affect the movement behaviour of rats.

3.5.2 Material and methods

The environment which was used in this experiment again consisted of two planes at a distance of 1 m to each other, which resembled the floor and the ceiling plane of the former experiments. These two planes were textured with the same gravel texture as in the previous experiment. We also used the same black and white striped columns with a diameter of 50 cm, which were arranged on a squared grid. The distance between the columns, however, was

enlarged to a value of 6 m for this experiment (Fig. 25). To present an optical flow field, we implemented a function in the experimental programme which allowed the experimenter to rotate the complete environment by pressing a button. The rotation was always centred on the actual position of the animal in the environment. The speed of the rotation was defined as 0.05 degree per frame, which resulted, at a frame rate of 50 Hz, in 2.5 degrees per second. The rotation was activated and deactivated by will of the experimenter. The animals and the rewarding procedure remained the same as in the previous experiments.

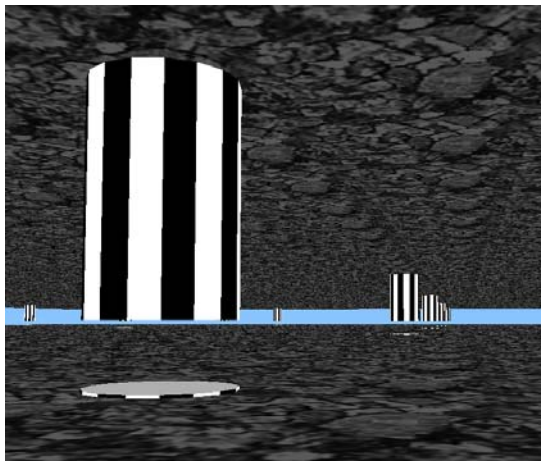


Fig. 25 Inside view of the environment in Experiment 7; column distance now enlarged to 6 m.

3.5.3 Discussion

The design of the environment turned out to be the crucial factor for this experiment, too. An immediate movement response connected with the onset of the rotation, as it was reported for the insects, could not be measured. Although we could see some rotations which corresponded to the environmental rotation, this rotations turned out not to be caused by the optic flow. As the incoherence with the onset showed us, this was due to an aiming behaviour of the animals when they tried to reach a target column. Following from this, we failed to present an appropriate optic flow environment to the animals. In their position the targets should have been independent from the optic flow stimulus, which they were not. Although we could not fulfil the intended goal of the experiment, we could observe some behaviour which was interesting with regard to the previous experiments. Apart from the optic flow, we challenged the animals with a rather difficult task. Although the target was constantly rotating away from their moving direction, the animals were still able to reach it on several occasions. This made clear to us that the animals really recognized the objects in the environment as such and were able to move towards them even under complicated conditions.

3.6 Experiment 8

3.6.1 Introduction

In the following experiment, our interests shifted towards the question if the behaviour of approaching a certain virtual object in a virtual environment can really be regarded as spatial behaviour. By that time, we had already seen that the animals were able run towards a target object quite successfully, but the question if this was just an aiming behaviour or if the animals perceived the environment and the elements in it as spatial remained still open. Some subjective observations, like on the afore-mentioned occasion when an animal turned around after closely missing a target, encouraged us in the belief that we were dealing with real spatial behaviour; however, the experimental prove for this remained to be delivered.

To address this question, a spatial task needed to be designed. One sort of clearly spatial behaviour is landmark navigation, i.e. a certain position is located by using the configuration of surrounding landmarks. Experiment 8 was designed to confront the animals with such a task.

3.6.2 Material and methods

The environment in this experiment was derived from the environment in Experiments 3-6 (Chapter 3.4). Two planes serving as ground and ceiling planes were distanced 2 m from each other and covered with the previously used gravel texture. In the centre, at 0/0 position, a target column was placed, where reward was provided in the same mode as described before. The target column was intended to function as a marker during the training to give the animals a direct visual cue. Later in the experiment, we planned to remove this cue and leave only the landmark information to the animals. As landmarks, we provided three 3-dimensional objects which floated above the ground in a distance of 50 cm. The first object was a sphere at position -2/0 with a diameter of 1 m. The second object was a cube of 1 m edge length at position 1/2.5 and the last object was a cone with a height and a base diameter of 1 m at position 0/1.5. The cone and the cube were black and white striped, whereas the sphere was covered with a black and white chequerboard texture (Fig. 26). We used the same starting points as in Experiments 3/4, to which we transferred the animal's position by pressing a button.

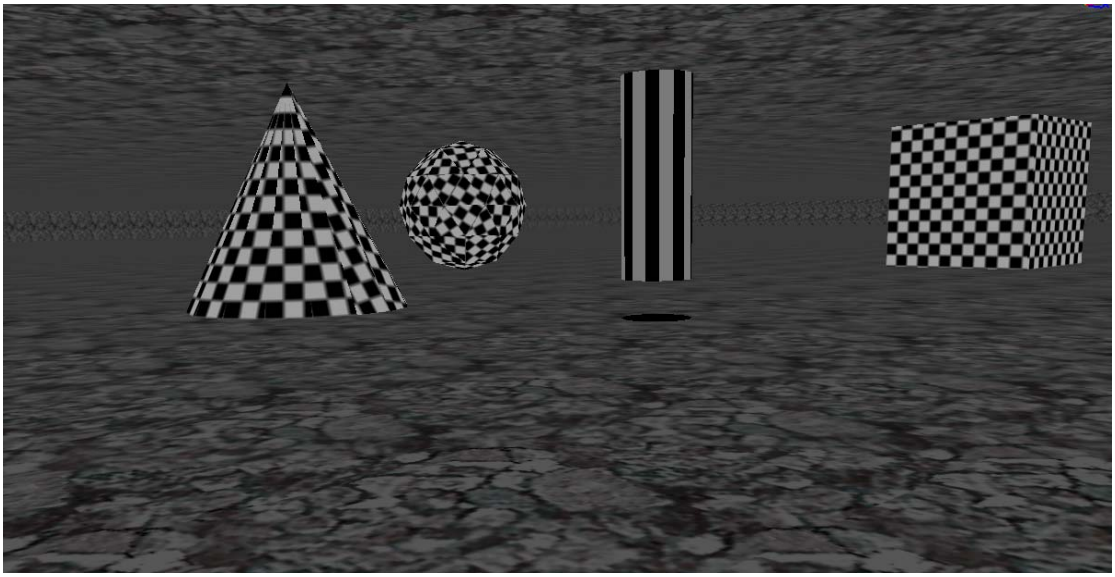


Fig. 26 Inside view of the environment in Experiment 8. The three objects surrounding the central column can be seen; the actual view height of the animals during the experiment was lower.

3.6.3 Discussion

In this experiment we learned that the reasons which had led to the failure of Experiments 3-6 were still persistent and, although we trained the animals for eight days in this environment, the tendency to run into a fixed direction was dominating enough to cover all other behaviours. Due to the rotating environment, no fixed direction had been observable in the previous experiment. Our hope that this behaviour was removed in Experiment 7 was not

fulfilled. It turned out that it is very persistent and that once it appears it is very hard to get rid of. The main lesson we learned from this experiment was that whenever an experimental task can be influenced by such behaviour, the environmental design must be altered in a way to prevent the emergence of such tendencies.

3.7 Experiment 9

3.7.1 Introduction

After the experiments which I have described so far we ended up with few successes but some promising approaches. We had also learned a lot about factors in the experimental design which can interfere with the task and therefore have to be prevented. The most promising experiment which we had realized at that point was Experiment 1; we thus decided to rerun this experiment. This included that we performed the experiment with a new set of naive animals (rat 161-171) which underwent the handling procedure described in the material and methods section. We also took care to exclude all the error sources we had encountered by then in order to create a virtual environment for this experiment. Experiment 9 is split into two parts a and b in which the only difference was that the distance between the columns was enlarged for Part b.
The results of this experiment were also published in the article: Hölscher et al. (2005).

3.7.2 Material and methods

The task for this experiment was again that the animals should run under a grid of suspended columns. Therefore, columns were placed in the environment, again, but they now had a diameter of 50 cm and were striped black and white. The distance from column centre to column centre and therefore for the whole grid was 2 m in Part a and 10 m in Part b. The floor and ceiling planes were textured with the gravel texture which was used in the previous experiments (Fig. 27/28).

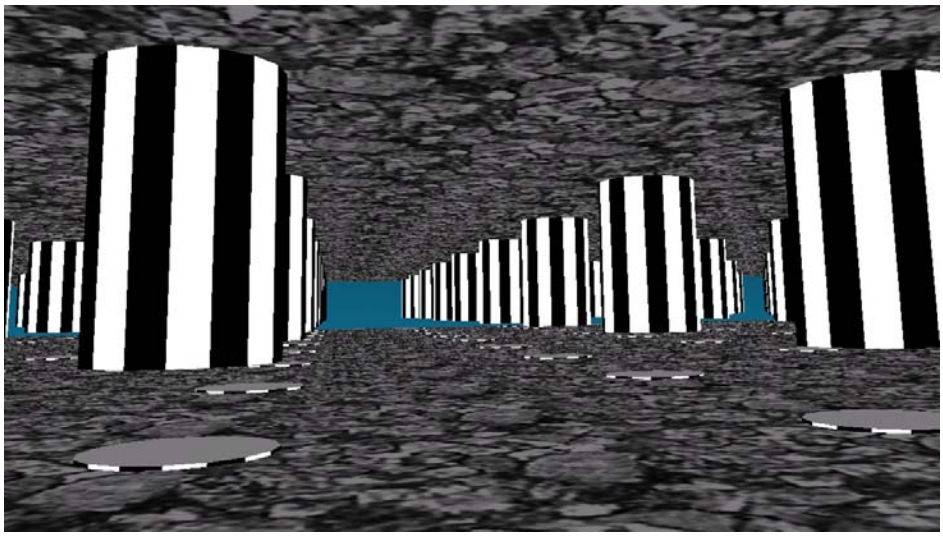


Fig. 27 Inside view of the environment in Experiment 9, with column distance 2m.

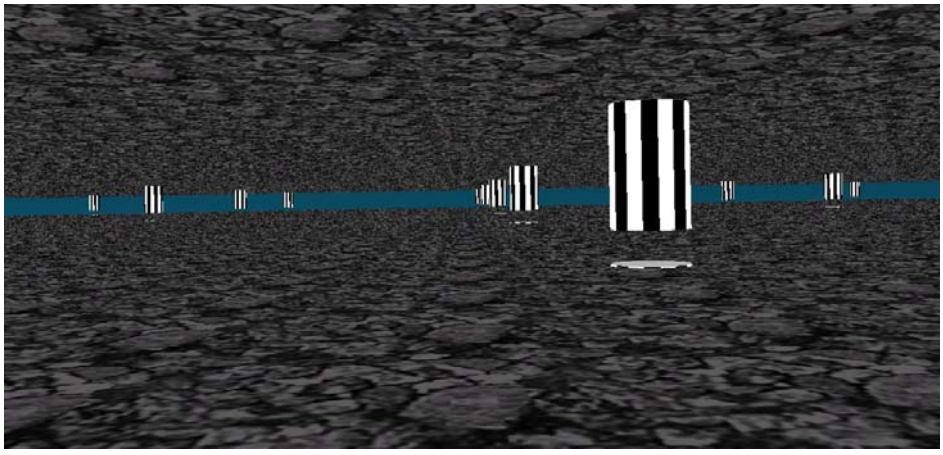


Fig. 28 Inside view of the environment in Experiment 9, with column distance 10m.

The rewarding procedure was similar to the previous experiments, as well. In this repetitive environment, the animals were allowed to run in any direction for the fix duration of 10 min. The sequence in which the animals participated in the experiment was randomized. Part a of the experiment lasted ten consecutive days and subsequently to this, Part b took place for another five consecutive days.

3.7.3 Results

Results of Part a with cylinder spacing of 2 m

As shown in Fig. 29 A–C, the animals improved considerably in their orientation to and in finding of the cylinders. The number of rewards (hits per 10 min run) increased steadily over the course of 10 days (one-way RM-ANOVA, $F_{9,11} = 40.7$, $P<0.0001$, post-hoc Bonferroni multiple comparison test, **P<0.01; ***P<0.001; N = 12; Fig. 29A). In Fig. 29B, the number of hits per 2 m distance increased over training sessions (ANOVA $F_{9,11} = 22.1$, $P<0.0001$, post-hoc test **P<0.01; ***P<0.001). While the ideal path would have followed a straight line between the cylinders (one reward every 2 m), the animals improved to an average of 0.76 rewards per 2 m. The path length per 10 min, i.e. the average speed of the animals, increased (Fig. 29C). The maximum path length per 10 min was 219.1 m.
The average path length over all 12 animals increased from 66.5 m to 166.2 m. Fig. 29D shows the inter-individual variation of the path length averaged over all 10 days. As a general feature, we observed that the animals tended to follow a more-or-less defined direction in the laboratory coordinate system over several days (Fig. 30A, 32A).This direction varied from rat to rat. Fig. 30 demonstrates in more detail how the path chosen by the animals improved over time. In Fig. 30A, the complete path of animal 9 for the first day (blue) and the tenth day (red) is displayed. The total path lengths were 70.6 m and 172.3 m, respectively. Fig. 30 B,C depict details of the paths boxed in Fig. 30A. Cylinders are drawn to scale as dark grey circles, hit cylinders are marked by a larger light grey circular surround. Dots on the path mark every 30th sample (about every 1.4 s). Cylinder spacing is 2 m. The much more regular path on day 10 as compared to day 1 is apparent; Fig. 30 B,C depict the most irregular part of the paths. The higher speed on day 10 can be inferred from the larger dot distance (Fig. 30B is at about twice the scale of Fig. 30C). The path on day 10 shows a more direct approach to the nearest cylinder. Fig. 30 E,G shows the angular histogram of the orientation of the animal's body long axis, i.e. of the angular incremental counter (aie) data. If the animal were to run along its body long axis from one cylinder on a straight path to one of the four nearest cylinders, we

would expect histogram peaks at N, E, S, W. On day 1 (Fig. 30E), we find no pronounced body orientation, while on day 10 (Fig. 30G), we find superimposed on a general orientation to SE, a peak orientation to S. In order to get a demonstration of the improvement of the 'cylinder hit probability' of our rats, we surrounded each cylinder by a 2 m * 2 m square divided into 7 * 7 subsquares. We counted the number of trace samples in each subsquare and added up the counts in corresponding subsquares (corresponding with respect to their position relative to the central cylinder).

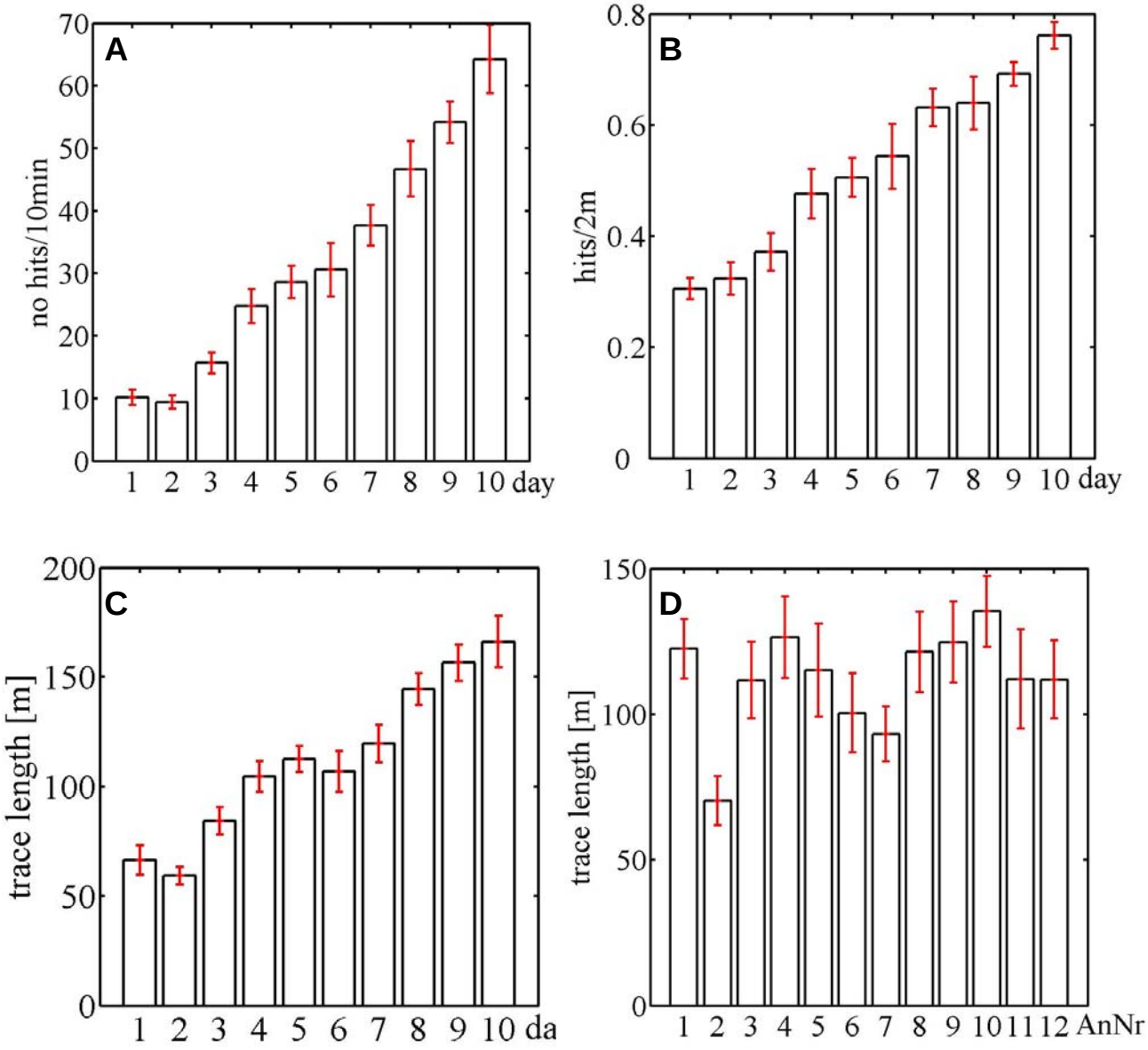


Fig. 29
A: The numbers of rewards (hits) per 10·min run increased over the course of 10 days (means ± S.E.M., N=12 animals; one-way RM-ANOVA, $F_{9,11}=40.7$, $P<0.0001$; post-hoc Bonferroni multiple comparison test, NS, non significant, **P<0.01; ***P<0.001; N=12). B: The number of hits per 2·m distance increased over time (ANOVA, $F_{9,11}=22.1$, $P<0.0001$; post-hoc test, NS, non significant; **P<0.01; ***P<0.001). The maximum number of hits achievable per 2·m was 1; animals reached a mean value of 0.76±0.024 (± S.E.M.). C: The average trace length per 10·min, i.e. the average speed of the animals, increased over 10 days. D: The inter-individual variation of the trace length, averaged over all 10 days, versus the animal number.

Fig. 30 D,F show the relative number of counts in corresponding subsquares. For the trace of a rat which does not aim at the cylinders, we expected an equal distribution of trace sample counts over all subsquares. The considerable increase of the 'trace density' under cylinders from day 1 to day 10 is apparent.

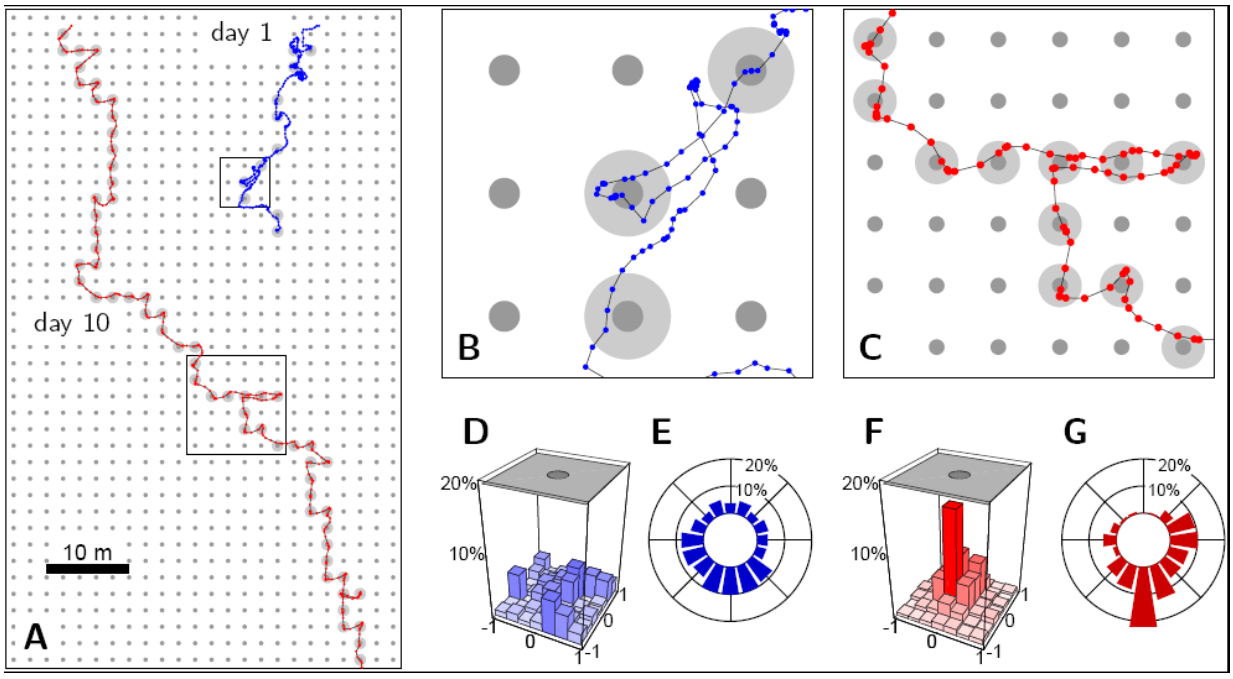


Fig. 30
Sample trajectories of day 1 (blue) and day 10 (red) for rat 9 in Experiment 2. A: Map of virtual environment with cylinders drawn to scale. Hit cylinders are encircled by light grey discs. Starting points appear towards the top of the figure. B: Detail of day 1 trajectory, as boxed in A. Dots mark every 30th trace sample (every 1.4·s). C: Detail of day 10 trajectory, as boxed in A. D: Rat average position relative to closest cylinder on day 1E: Histogram of body orientations on day 1 as measured using the angular incremental encoder. F: Rat average position relative to closest cylinder on day 10. Note the much more peaked distribution, indicating that the rat spent more time in the vicinity of the cylinder than on day 1. G: Histogram of body orientation on day 10. Note the pronounced orientation to S on day 10 compared to E, superimposed on a broadly distributed general orientation to SE.

Results of Part b with cylinder spacing of 10 m

Experienced rats from the previous experiment were transferred to a V.E. with cylinder spacing of 10 m and a radius of 2 m. The results are shown in Fig. 31, 32, using the same conventions as in Fig. 29, 30. In Fig. 31A, the number of rewards (hits) per 10 min run increased over the course of 5 days (oneway RM-ANOVA, $F_{4,11} = 7.3$, $P<0.0001$, post-hoc Bonferroni multiple comparison test, *$P<0.05$; **$P<0.01$; ***$P<0.001$; $n = 12$). In Fig. 31B, the number of hits per 10 m distance run also increased over time (ANOVA, $F_{4,11} = 5.37$, $P<0.002$, posthoc Bonferroni multiple comparison test, **$P<0.01$). The maximum achievable number of hits per 10 m was 1; our animals reached an average value of 0.644. The average path length of 12 animals increased from 154.1 m to 176.3 m per 10 min (Fig. 31C), the maximum path length was 228.8 m. Fig. 31D shows the inter-individual fluctuation of path length averaged over 5 days. In Fig. 32A, the whole trace of animal number 9, the same as in Fig. 30A, is displayed for day 1 (blue) and day 5 (red). The trace lengths are 123.7 m and 214.4 m, respectively. Fig. 32 B,C show the boxed details of these traces. The traces appear, as compared to Fig. 30C, slightly less precise in their orientation to the nearest cylinders as

demonstrated in the angular histograms of Fig. 32 E,G. The traces appear, as compared to Fig. 30 C, slightly less precise in their orientation to the nearest cylinders. In Fig. 32 D,E, we surrounded each cylinder by a square of 10 m * 10 m and subdivided it into 7 * 7 subsquares. The relative trace sample density is very high in the subsquare under the cylinder (Fig. 32 D,E).

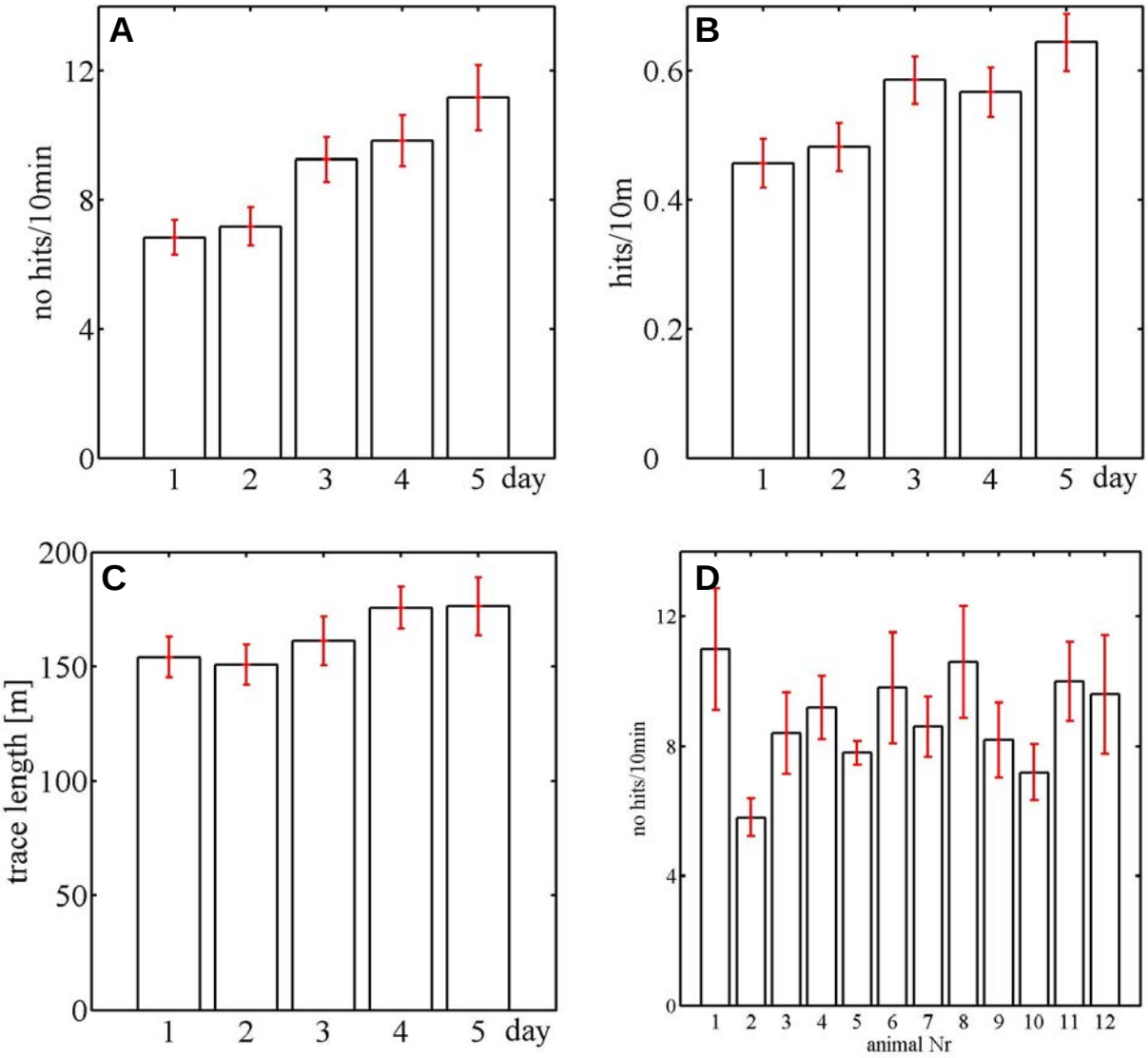


Fig. 31
A: The numbers of rewards (hits) per 10·min run increased over the course of 5 days (ANOVA, F4,11 = 7.3, P<0.0001; post hoc test, NS, non significant; *P<0.05; **P<0.01; ***P<0.001; N = 12). B: The numbers of hits per 10·m distance run increased over time (ANOVA, F4,11 = 5.37, P<0.002; post-hoc test, NS, non significant, *P<0.05; **P<0.01). The maximum achievable number of hits per 10·m was 1; animals reached 0.644. C: The average trace length per 10·min increased slightly over 5 days; the animals were trained in experiment 1. D: The inter-individual variation of the trace length, averaged over all 5 days, versus the animal number.

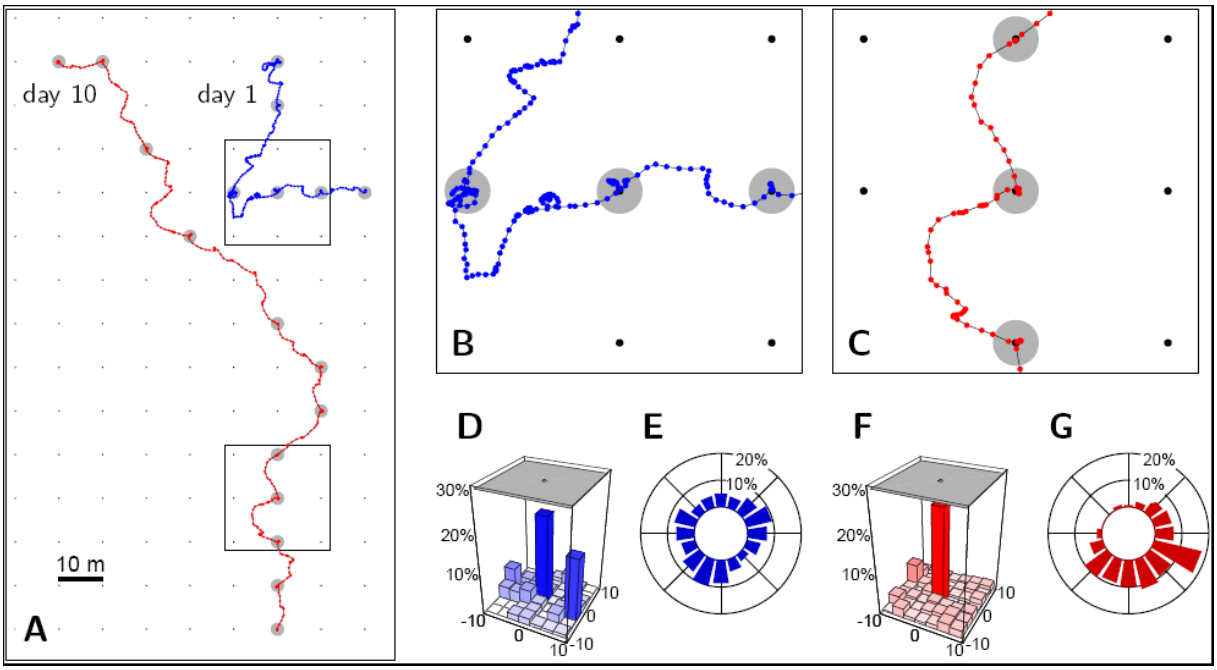


Fig. 32
Sample trajectories of day 1 (blue) and day 5 (red) for rat 9 in Experiment 3. A: Map of virtual environment with cylinders drawn to scale. Hit cylinders are encircled by light grey discs. Starting points appear towards the top of the figure. B: Detail of day 1 trajectory, as boxed in A. Dots mark every 30th trace sample (every 1.4·s). C: Detail of day 5 trajectory, as boxed in A. D: Rat average position relative to closest cylinder on day 1. E: Histogram of body orientations on day 1 as measured using the angular incremental encoder. F: Rat average position relative to closest cylinder on day 5. G: Histogram of body orientation on day 5. Note the dominant orientation to ESE on day 5 compared to the orientation peak to S in day 1.

3.7.4 Discussion

These results provided for the first time unquestionable data which prove that rats are able to navigate in virtual environments and to interact with the objects in it. The animals developed the two well known strategies at the beginning of the experiment. One group was running along rows of cylinders and sooner or later developed a fixed direction, whereas the other group followed a circling strategy. These circling strategies were reduced by excluding an immediate second reward under the same cylinder. Most animals appeared to prefer a fixed running direction with respect to the laboratory coordinate system later on in the experiment. This direction varied from rat to rat, but was maintained over several sessions.
The time required and the distance run to obtain rewards continuously shortened during training, suggesting that the animals developed a strategy to optimise their foraging behaviour Within the movements along that general direction, most rats clearly noticed the landmarks and navigated towards them. In the 2 m cylinder spacing Part a, the rats could easily see the four nearest cylinders from under the cylinder where it had just been rewarded. The cylinders covered an azimuth of about 14° and an elevation of about 22°. On day 10, many rats ran for a large part of their trace on a straight path from one cylinder to the next (see Fig. 30 A,C). The number of hits per 2 m trace length reached the average level of 0.76, which should be compared to the maximum possible value of 1. In contrast, in the 10 m cylinder spacing Part b, the nearest cylinders covering 4.6° of elevation and 2.9° of azimuth were not that easily visible to the rats. As a consequence, traces are not so well oriented towards the nearest cylinders (see Fig. 32 A–C). The animals did not quite reach the level of efficiency which they achieved in the experiments with 2 m cylinder distance. They hit fewer cylinders per 10 m trace length (0.64 compared to the possible maximum of 1). However, when the

cylinders became more visible, rats did turn towards them, found them and got a reward. The relative frequency of trace samples in the vicinity of cylinders shows a pronounced peak in the subsquare below the cylinder (see Fig. 32 D,E). The traces never became 'optimal'. There seems to be a tendency to deviate from the best path even when goals are clearly visible (see Fig. 30C). Perhaps we may interpret this 'noise' as an investigatory strategy that cannot be suppressed. Since there are no repetitive and predictable natural environments with a 100 % reward probability, it is of adaptive value to constantly deviate slightly from the seemingly perfect strategy in order to increase the likelihood of finding new food sources which otherwise would have been missed by running past them.
Despite this little interferences, Experiment 9 was by far the most successful experiment we had performed until then and revealed the results we had expected from the beginning without any of the disturbing influences which had spoiled the preceding experiments. The main reason for its success is, in my opinion, that the animals we used in this experiment were naïve animals which were not influenced by experiences from preceding tasks.

3.8 Experiment 10

3.8.1 Introduction

In continuation of the previous experiment, the focus of our interests was again led towards the elimination of doubts about the spatiality of the observed animal behaviour. We decided to create a task which challenged the animals to consider the spatial configuration of the elements within an environment to successfully reach a target. With the results of Experiment 8 in mind, we did not use an invisible target and tried to avoid other distracting elements. A very important point also was to avoid that the animals develop fixed directions. Unfortunately, we failed in meeting this demand, for which reason we readapted the settings twice, with increasing focus on this problem. To display the procedure which led us to the following series of experiments, I will present them in their order of introduction, combined with the considerations which led to them.

3.8.2 Material and methods 1

Imbedded in our standard ground and ceiling planes with a gravel texture and a distance of 1 m to each other, three columns were placed in a way that they formed an isosceles triangle (Fig. 33). Each of the columns at the corners of the triangle was at a distance of 2.5 m from the starting point at 0/0. By placing the columns at the position 1.5/-2, -1.5/2 and 0/2.5 we achieved, seen from the starting point, a group of two columns on one side and a single column on the other side. Only the single column at position 0/2.5 was rewarded and, due to the fact that the animals should discriminate them by using the spatial configuration, they were all equally black and white striped, as in the previous experiments. When the animal received a reward, a shift procedure was initiated comparable to the same event in Experiments 3-6. In contrast to these experiments, the procedure was triggered automatically without any influence of the experimenter. The shift event was also triggered by a second condition which occurred when the animals moved farther than 10 m away from the starting point at 0/0. To mark the shift event clearly, the environment became completely white for

three seconds. During the shift events, a second action was performed by the program. To avoid fixed directions, a function was introduced which generated a random multiple of 90° by which the environment was rotated around the starting point. The rotation was intended to force the animals to focus on the configuration and to follow the virtual cues towards the target. For this experiment rats 161-171 were used, again.

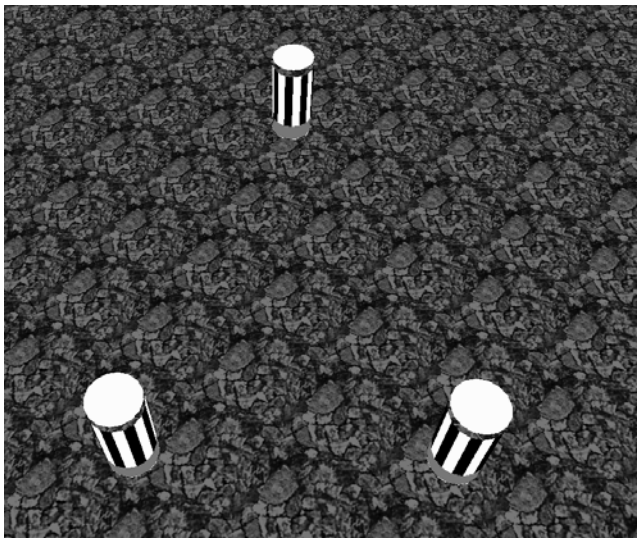


Fig. 33 Top view screenshot of the environment of Experiment 10; the arrangement of the columns in the shape of an isosceles triangle can be seen.

3.8.3 Results and discussion 1

After eight days of experiment in the above described environment, we could derive from the data that the rate of successful hits did not differ clearly from the chance level of 33.33 % and did not show a tendency to increase furthermore. By a closer look on the trajectories of the animals, we could observe that, in spite of all our precautions, a fixed direction had been established in a varyingly strong occurrence in all animals. The trajectories can be drawn in relation to the fixed lab coordinates, as in (Fig. 34A), then the fixed direction of the animals becomes obvious, whereas the positions of the columns which are marked by a cross in case of a hit are at varying positions. In contrast to this, the trajectories can also be drawn in relation to the rotated environment, which causes the column positions to remain stationary, whereas the trajectories of the animal point into varying directions (Fig. 34B).

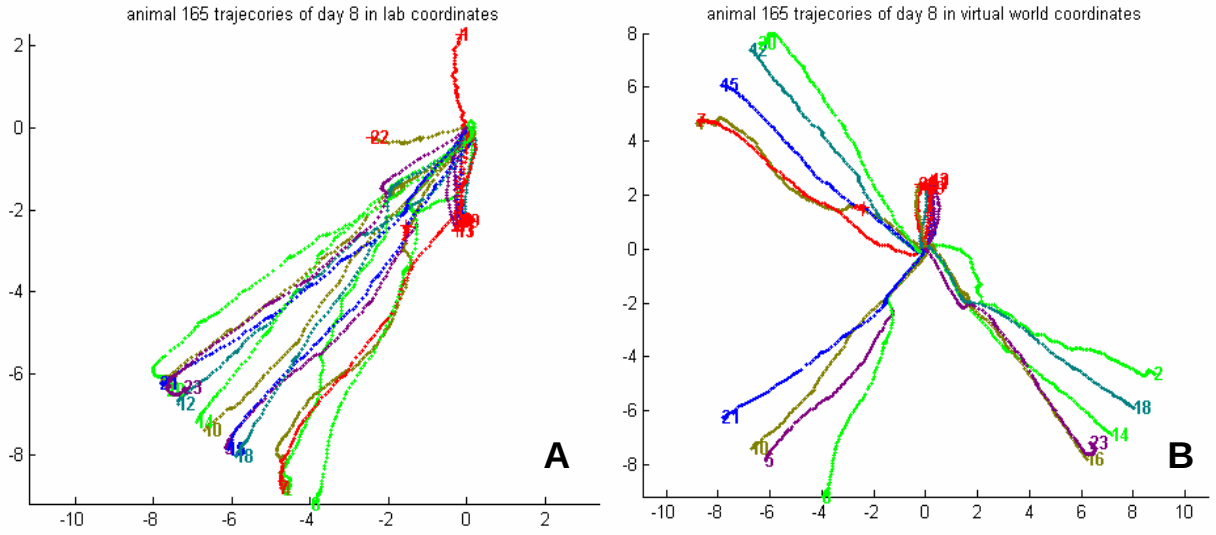


Fig. 34
Displays two views of the same example trajectory from the first experimental procedure. A: trajectory in lab coordinates; the orientation towards a fixed direction is evident. B: the equivalent data plotted in virtual world coordinates in which the targets are stable and the positions of the triangle can be seen in the centre were the successful runs end. The single trials are plotted in varying colours to make them better distinguishable.

When one compares these trajectories, a certain strategy can be recognized. When following their fixed direction, the animals mostly managed to reach a column when it was in a catchment area of approximately +/- 45° of their movement direction. Beyond that angle, they do not seem to be willing to deviate from their moving direction. By rotating the environment, we randomly positioned one of the columns into this catchment area, which caused the percentual hit rate to range close to chance level.
As possible reasons for this immediate occurrence of fixed directions, various factors have been discussed. One argument was the fact that the new setup confronted the animals with a great variety of new elements in the experimental procedure, such as the shift sequence, the rotation and the task itself within the configuration. In our opinion, the failure was based on the fact that the elements had not been introduced step by step and therefore too much information had to be learned at one time. For this reason, we decided to simplify the experiment for a certain training time and to come back to the original experiment later.

3.8.3 Material and methods 2

The main feature which was removed for the training environment was the shift sequence after a column hit. To keep the animals in the centre of the environment without replacing them, the arrangement of the columns was modified, too. Instead of three columns, six columns were now arranged in a hexagon around the starting point, with an equal distance of 3 m between each other and to the starting point. This setting should lead the animals to run from column to column in circles and, by doing so, get rid of the fixed direction. With this change, two of the above mentioned new elements were removed to provide a fairly simple task for the animals which was somehow more related to the task they had performed in the previous experiment. For the case that an animal missed a column or walked straight on after a successful hit, we had to prevent them from departing too often from the column area. For this reason, we could not completely resign from the shift sequence and activated it after the animal was more than 15 m away from the starting point.

3.8.4 Results and discussion 2

After nine successive days, the experiment was again interrupted since we were not able to force the animals to perform circle runs on the column hexagon with the above prescribed setup. The best performance we could achieve is displayed in Fig. 35A. For most of the other animals, it turned out that they were not willing to deviate from their actual moving direction after a target hit and therefore continued straight on until the shift sequence transferred them back to the starting point. This behaviour was prominent in every animal at the beginning of the training (Fig. 35B), but we expected that the reduced reward, due to the extended sojourn away from the column area, should force them to stay in the centre. Except from the above displayed animal, our expectations were not fulfilled. Fig. 35C is a more representative example of the performance of the majority of the animals. Not only did they, apart from some few occasions, refuse to run from one column into the direction of the next column, but they also kept on running into a fixed lab direction. After this finding, we decided
that a continuation of the training was futile.

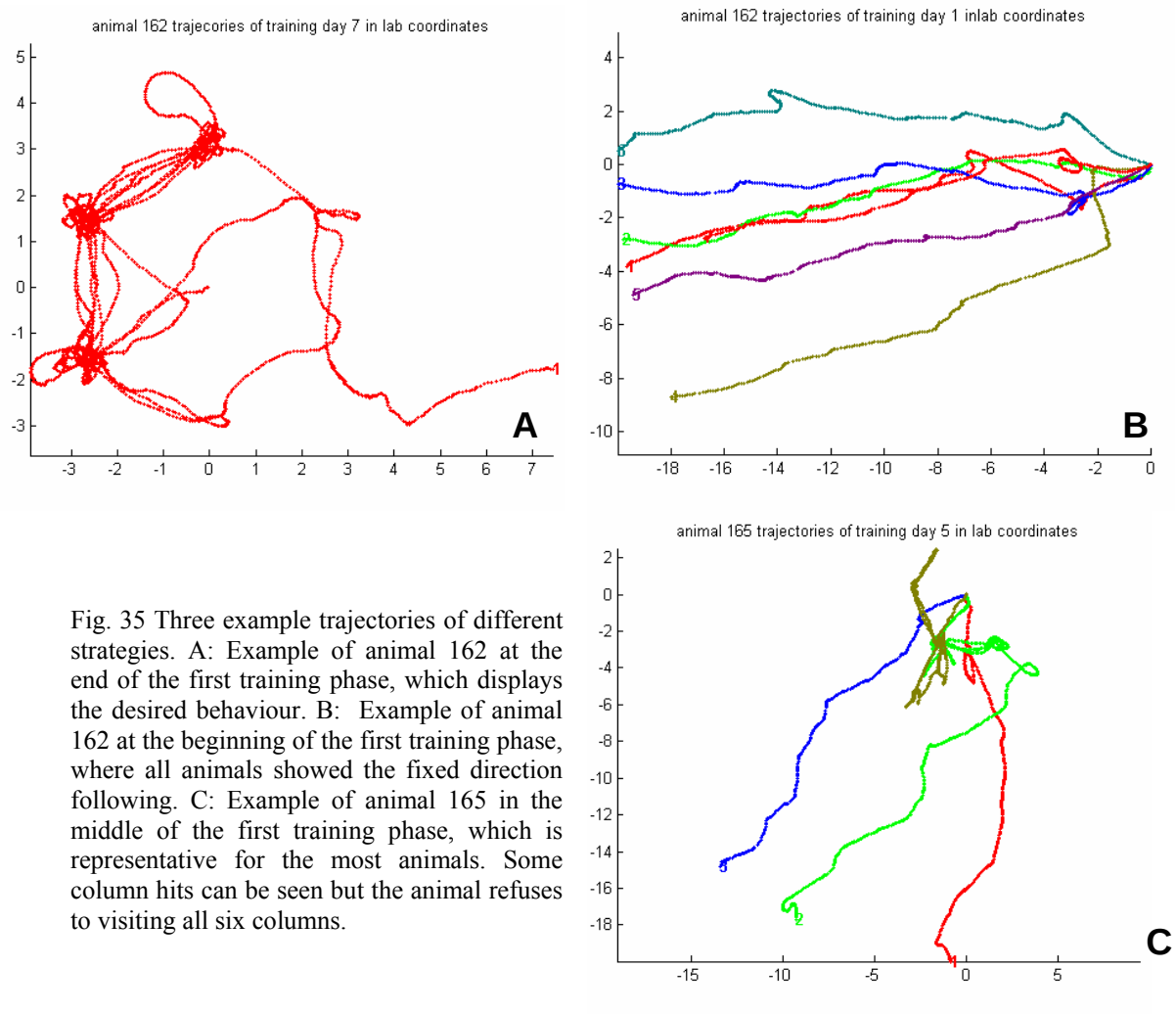


Fig. 35 Three example trajectories of different strategies. A: Example of animal 162 at the end of the first training phase, which displays the desired behaviour. B: Example of animal 162 at the beginning of the first training phase, where all animals showed the fixed direction following. C: Example of animal 165 in the middle of the first training phase, which is representative for the most animals. Some column hits can be seen but the animal refuses to visiting all six columns.

3.8.5 Material and methods 3

To address the problem of the fixed direction, we decided to use the behaviour which the animals already showed and created the environment in a way that we could modify this behaviour so that these directions might become eradicated. So far, all animals had shown that they could run reliably from the starting point to a column which lay within the catchment area of their fixed direction. By reintroducing the shift sequence, we could lead the animals repeatedly to do this. However, while doing so, we rotated the environment after every hit by just 10°. With these slight rotations, we hoped that we were able to "drag" the animals away from their fixed directions. On the first day of the following training, we started with four columns equally distanced from the starting point. On the second day, the number of columns was reduced to two and finally, on the fourth day, only one column was left in the environment which was used for three more days.

3.8.6 Results and discussion 3

It soon turned out that with the four-column configuration the animals kept running reliably towards a rotated column. But, as mentioned above, the animals seem to have a caching area of approximately +/- 45°, therefore the animals could stick more or less to their fixed direction within this catchment area and, whenever a column was rotated outside this range, another one re-entered the area on the other side. Fig. 36A displays an example trajectory of this behaviour, where the animal is repeatedly moving within such a 90° quadrant.

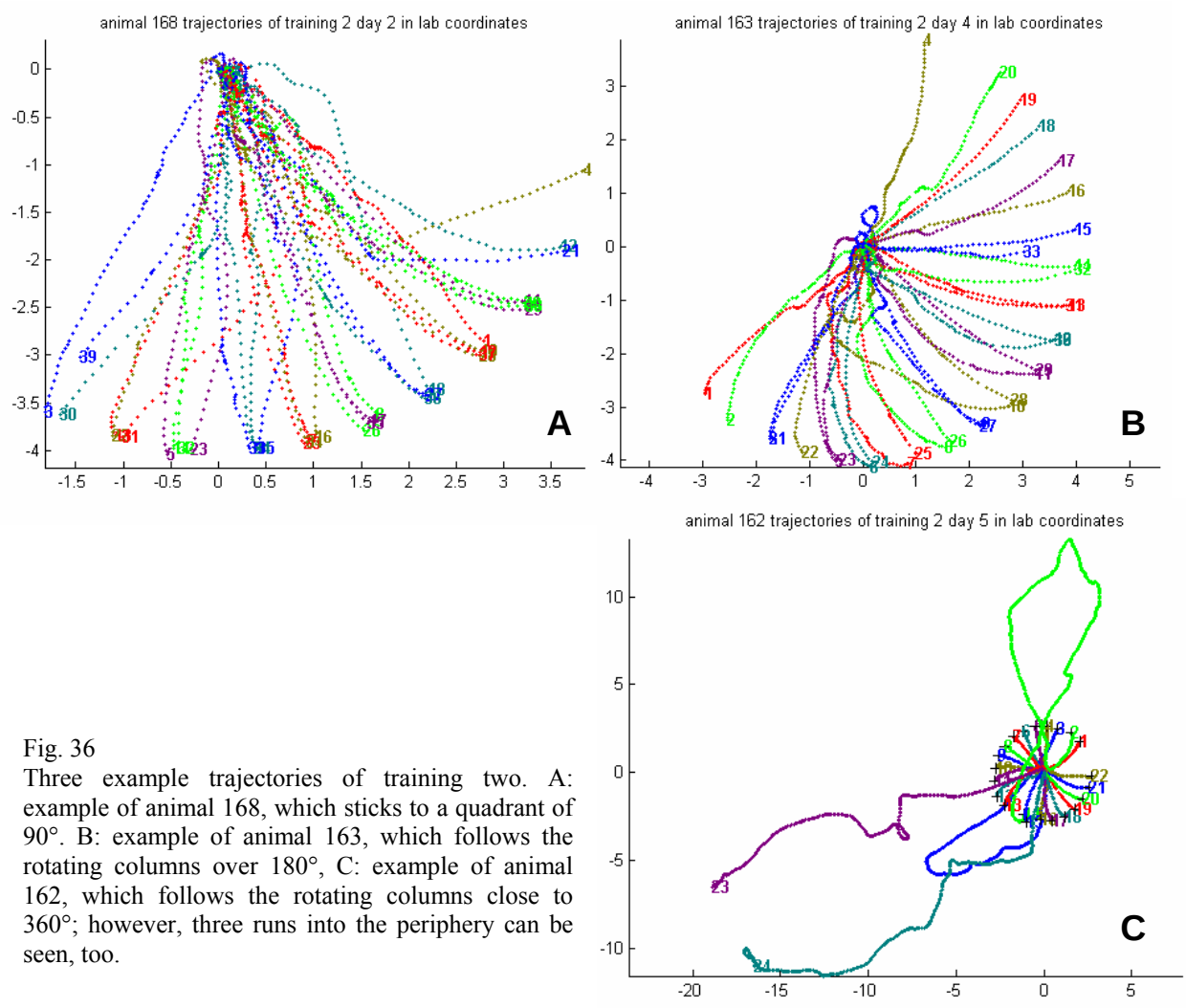


Fig. 36
Three example trajectories of training two. A: example of animal 168, which sticks to a quadrant of 90°. B: example of animal 163, which follows the rotating columns over 180°, C: example of animal 162, which follows the rotating columns close to 360°; however, three runs into the periphery can be seen, too.

To force the animals to go beyond this quadrant, only two columns were used, so that the animals had to follow the column for at least 180° to receive a reward. Some of the animals were able to do this, as one can see in Fig. 36B, but it also occurred more often now that the animals performed unsuccessful runs into the periphery when the columns were too far away from their catchment area. This happened even more often when we reduced the number of the columns to one. We could extend the catchment area of some animals up to 180° and in one single case even beyond this amount (Fig 36C), however, none of the animals was able or willing to follow the column for a complete 360° rotation. These directions had already been established during Experiment 9, when we did not try to prevent their occurrence. This demonstrated to us that following a fixed direction is a behaviour or strategy which, once it is established, is very hard to remove and therefore influences all performances.

3.9 Experiment 11

3.9.1 Introduction

With respect to the outcome of Experiment 10, we asked ourselves if we could continue experimenting with the animals although they showed this persistent fixed direction. Therefore we decided to create a task with which we could use the behaviour which the animals already showed. Even tough they followed their fixed direction, Experiment 10 had shown that they responded to targets within a certain catchment area, so we thought that by presenting at least two different targets within this area, we could create a discrimination task. Beside the experiments on high-level cognitive behaviour, such as spatial navigation, we also had to clear some basic properties of our setup. Apparently, one can assume that the psychophysical properties in our setup differ from the real world. Therefore we thought it was good to know the minimal contrasts and spatial distances which the animals are able to discriminate in our setup. The spatial discriminability is a function which also varies according to contrast. To keep the tasks simple, we decided to investigate these two factors independently and concentrated on the contrast function first. Before, however, the procedure for a successful discrimination task had to be created. To accomplish this, our experiences from Experiment 2 and Experiment 10 needed to be considered.

3.9.2 Material and methods

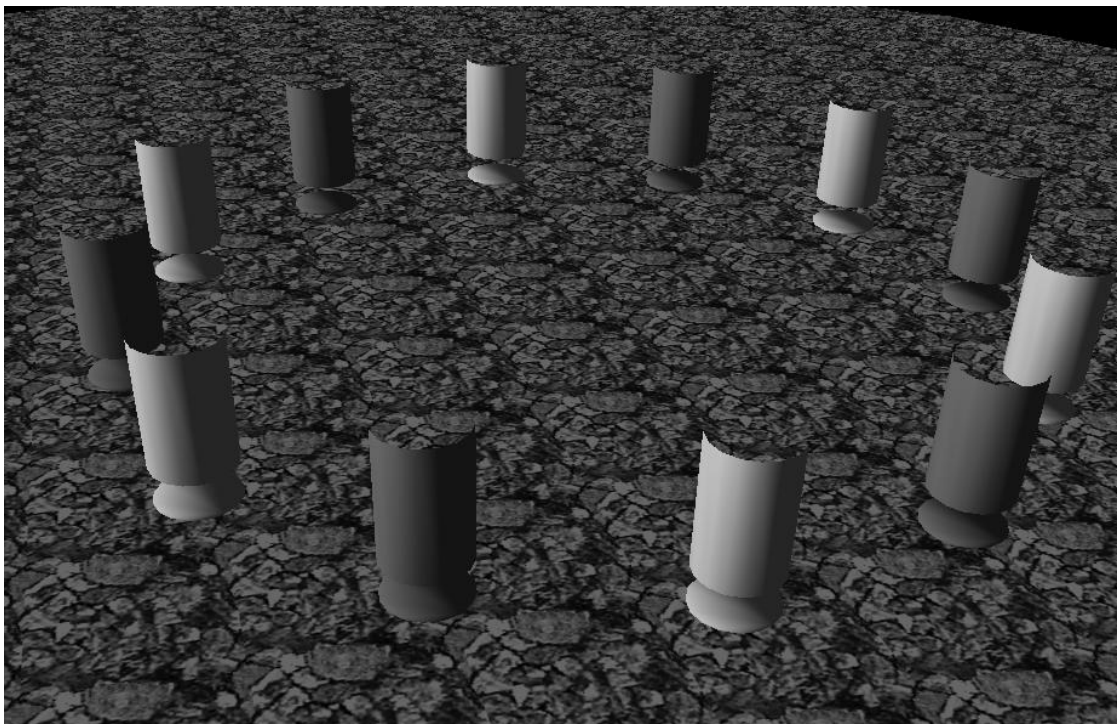


Fig. 37 Half top view screenshot of the environment in Experiment 11. The circular arrangement of the columns can be seen, only the visible part of the columns is rendered in this view, unlike in the experiment were the objects are completely rendered.

The environment was similar to the one which was used in Experiment 10. The two environments differed only in the number of columns and in their colouring. 12 Columns, six light grey and six dark ones, were placed on a circle with a radius of 3 m around the starting point. This resulted in a distance of 1.5 m between the centre of each column or 30°, seen from the starting point. With a diameter of 50 cm, the columns had an angular size of 10° at the starting point, following from this, the gap between each column had a size of 1 m or 20°. The two colours were arranged in alternating order on the circle. The colour intensities were chosen as 90 % of plain white for the light grey columns, 60 % for the dark grey columns and 30 % for the background (Fig. 37). The procedure of the experiment conformed to the procedure of the final part of Experiment 10. Beginning from the starting point at 0/0, the animals could run towards the columns which surrounded them. When they managed to run underneath one, the environment turned white for 3 sec and the rats were shifted back to 0/0. When they did not hit a column, the same shift sequence was initiated when the distance to 0/0 exceeded 10 m. Simultaneously to the shift sequence, a rotation of the environment was performed to make sure that the animals used the visual cue and did not run continuously into the same direction after they had once received a reward. As an appeal for the animals to discriminate between the two types of columns, only the bright grey coloured columns were rewarded (day 1-4). In a second part of the experiment, the rewarding was given at the dark grey columns. Animals 160-171 participated in this experiment for 10 min each day over 57 days.

3.9.3 Result and discussion

From the first day on, the performance of the animals was significantly over chance level ($p<0.001$ individually t-tested against a chance level of 50 %) for the four days on which the bright grey columns were rewarded.

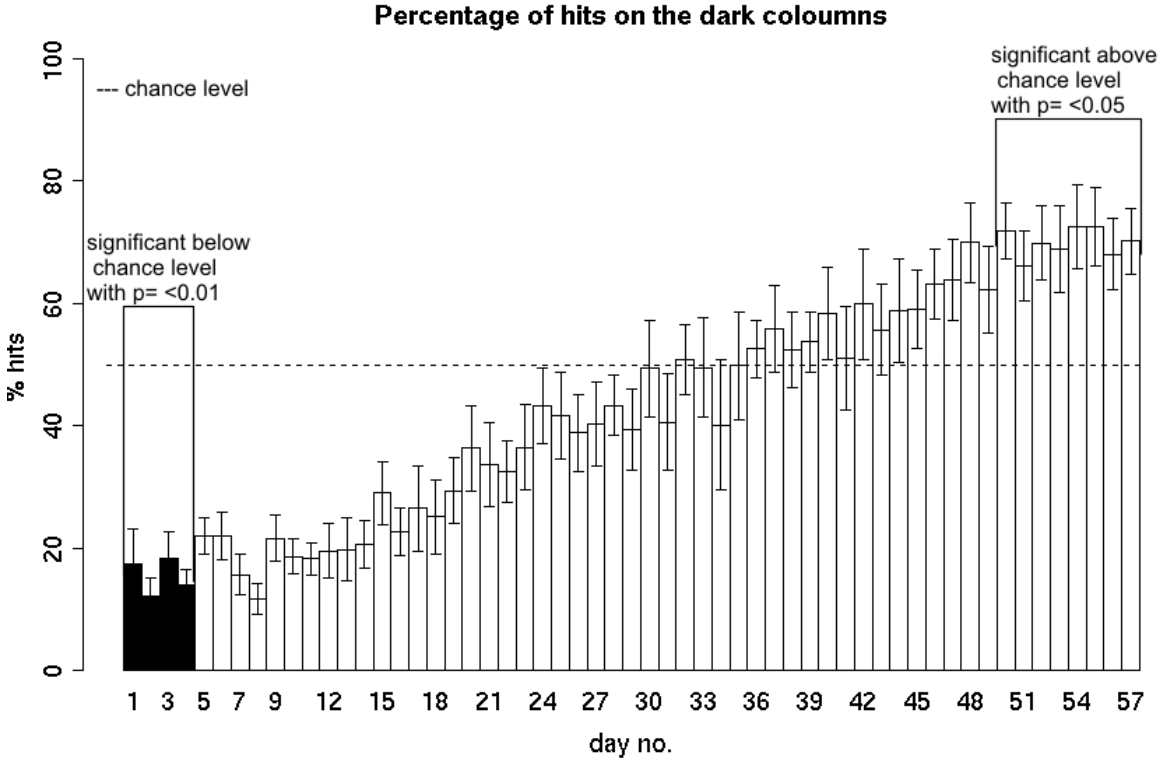


Fig. 38 Display of the percentage of hits on the dark columns over all experimental days. Black bars represent the days during which the bright columns were rewarded; white bars represent the days during which the dark columns were rewarded.

Strangely enough, this performance appeared immediately and no learning phase could be observed. To control whether this behaviour was driven by the reward, it was relocated to the other type of columns. After this change, it immediately became clear that an instant preference for the bright columns had motivated the animals. Fig. 38 displays the percentage of hits at the dark columns over all experimental days. The black bars represent the four days on which the bright column was rewarded, whereas the white columns represent the following days on which the dark columns were rewarded. With more that 80 % on the first four days, the hit rate lies highly significantly over the chance level of 50 %. On the following days, however, the animals continued persistently to run towards the bright columns although the dark columns were rewarded now. This can only be explained by the fact that the animals had a strong preference for the bright columns. One could assume that the animals were probably not able to see the dark columns at all. However, as a counter argument we could observe that, on the few occasions when the animals did not run towards a bright column, they were running towards a dark one and not into the periphery, for which the probability would have been much higher. We assumed that this preference had probably something to do with the contrast against the background. On the following days, the preference proved to be a strong one; even with the reversed reward it took about 9 days until the percentage of dark columns started to increase, but still then we could only see a very slow increase. The learning phase which we had missed in the beginning started to take place then. Finally, after 46 days of learning the percentage of rewarded columns was for the first time significantly over chance level ($p<0.05$, individually t-tested against a chance level of 50 %). On the following seven days, the performance increased again slightly until, during the last three days, a p value of over $p<0.01$ (individually t-tested against a chance level of 50 %) could be reached, which was sufficient to terminate the experiment. We had been able to demonstrate that the animals are able to discriminate between the two types of columns which differ only in their brightness level. But we had also learned that a difference in brightness can induce very strong preferences which can severely interfere with the experimental process. This experiment was pursued as subject for the diploma thesis of Jan Regler (2006). He could demonstrate that the contrast against the background plays a big role for the observed preferences, but he also found out that there are further factors which influence the direction of these preferences. He was also able to measure a psychophysical function for the contrast perception in our virtual setup.

3.10.0 Foreword to Experiment 12-a and -b

Since the animals had shown such a persistent fixed direction in the previous experiments, Experiment 12 began with the introduction of new animals. 12 male Long Evans rats, numbered 646-657, were split into two groups (12-a = 646-651; 12-b = 652-657). To avoid that a complete group of animals was spoiled by a deficient environment, we decided to try out two different approaches with two smaller groups and to rerun the more promising one afterwards.

3.10 Experiment 12-a

3.10.1 Introduction

In Experiment 12-a, we wanted to figure out a method which allowed us to present a maze-like environment to the rats although we could not simulate walls. With such a method, we would have been able to construct a task comparable to the big variety of existing real world experiments of which the majority takes place in mazes. One way to achieve this could be to elicit a street following behaviour. We assumed that if we were able to train the animals to follow stripes of a certain distinguishable pattern, we could create all sorts of mazes with this type of stripes, which would allow us to simulate several real world experiments.

3.10.2 Material and methods

The ground and ceiling plates were again covered with the gravel texture and distanced 1 m apart. To have enough explorative room, a radius of 100 m was chosen. The streets that were placed within this environment were built of 1 m wide stripes which were textured with a black and white checker pattern. Since virtual planes have no height, they are hard to see from a certain distance. To enhance the visibility of the street pattern from greater distances, we suspended cubical-shaped blocks of the same dimensions and position as the street stripes from the ceiling. The height of the blocks was 50 cm, which led to a distance of also 50 cm from the street to the lower side of the block. An inside view of such a street environment can be seen in Fig. 39. To accustom the animals slowly to the street principle, we created an environment in which four streets crossed each other with an angle of 45° at the position 0/0 and stretched until the edge of the ground plate, which made them 200 m long.

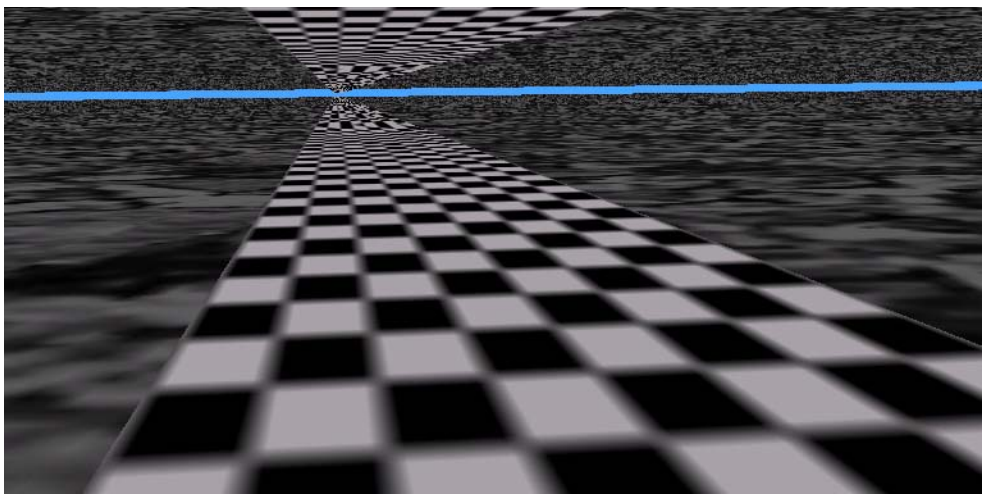


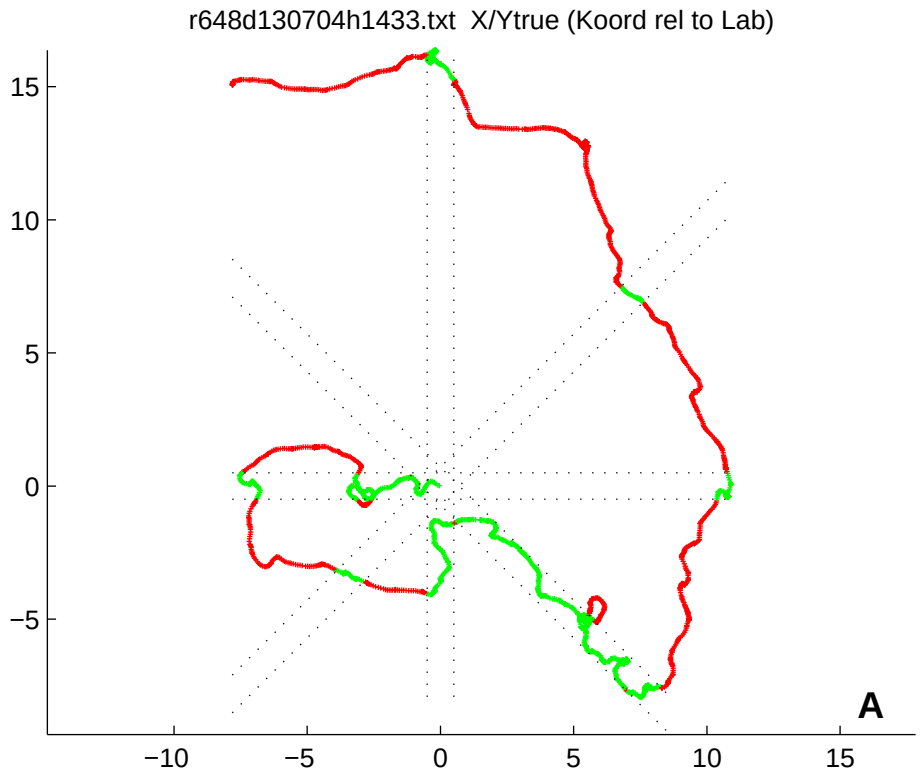
Fig. 39 Inside view of the first environment in Experiment 12-a, which displays the view along one street branch away from the centre; this image approximately renders the view height of the animals during the experiment.

Starting in the centre of this environment, the animals crossed the streets on many occasions when randomly exploring the environment. Every time they entered the pattern of a street at any given position, the animals received a reward. For every following 50 cm they continued moving inside the street pattern, another reward was applied. As in the previous experiments, the rewarding procedure was accompanied by the sounding of the “ding“.wav file. In the case that the animals left the street pattern, the “ring” sound file was played and repeated every meter they moved further outside the streets. This initial environment was modified in small

steps as soon as the animals' performance achieved a level which allowed the next step to take place. As a first step, the number of streets was reduced from four to two, and subsequently to one street in a second step. Finally, only half a street beginning at point 0/0 was left. After the animals had learned to orient themselves reliably towards this street and to follow it, we enlarged the rewarding distance first to 1 m and later to 2 m. Since we could also observe that the animals tended to turn around after a few meters, we also introduced a direction dependent reward.

3.10.3 Intermediate results

In Fig. 40, several stages of the experiment are displayed with an example trajectory. The lines dotted in black mark the borders of the streets. Fig. 40A is an example from an very early stage at which four streets were still crossing in the middle, and one can see that the animals hit the streets in a rather random fashion. The next image (Fig. 40B) shows an intermediate phase of the training. On some occasions, the animals followed the streets for a certain period, but it often occurred that they deviated from the street after a certain time, but re-entered it short after. The final performance we could observe is displayed in Fig. 40C, in which only one road is left and the reward is only given while the rats move towards the periphery. After all animals had reached this level, which also meant that over 90 % of their trajectory was on the street, we were confident that we could continue with this paradigm and try to introduce more challenging tasks. The training lasted 26 days in the multiple road environment and another 20 days in the single road environment to achieve the presented performance.



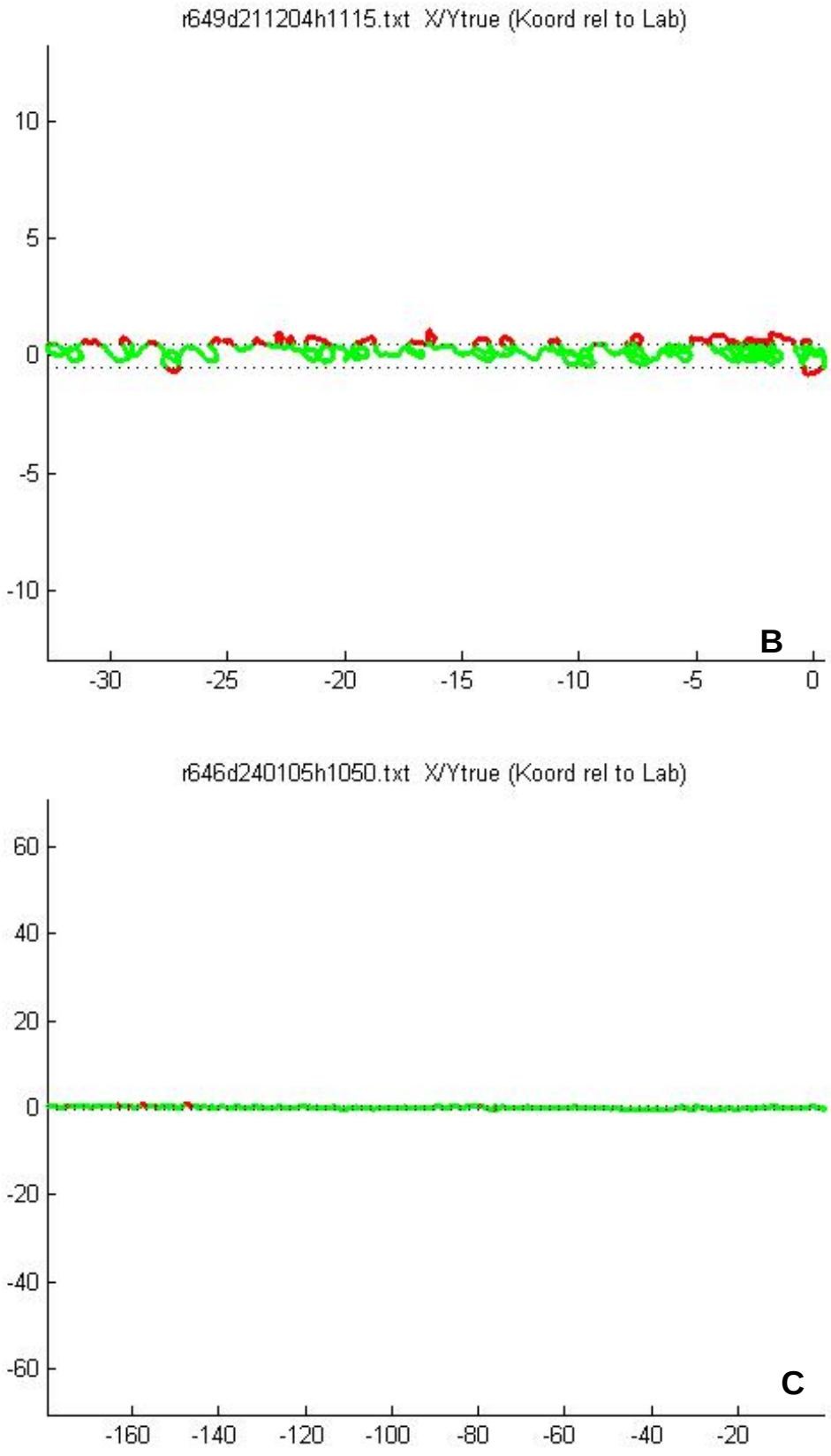


Fig. 40
Display of three example trajectories from the training procedure. The trajectories are coloured green when the animal's position was on the street, and coloured red when the animal was off the street. The black dashed lines mark the positions of the streets.

3.10.4 Material and methods 2

Having the street following behaviour at hand in an appropriate fashion, we planned to use this to confront the animals with a task which required spatial navigation behaviour. For this purpose, a new repetitive environment was created which contained streets like those in the above mentioned environments. The arrangement of the streets in this environment was a honeycomb pattern. The edges of the hexagons had a length of 10 m. The streets themselves were created with the same textures and measures as in the previous experiment. To prevent the animals from getting overwhelmed by to many novelties, we started with some habituation days on which the streets were rewarded in every direction and with a high frequency (every 50 cm). We planned to introduce landmarks later which should guide the animals onto the right branch of a Y-crossing (Fig. 41).

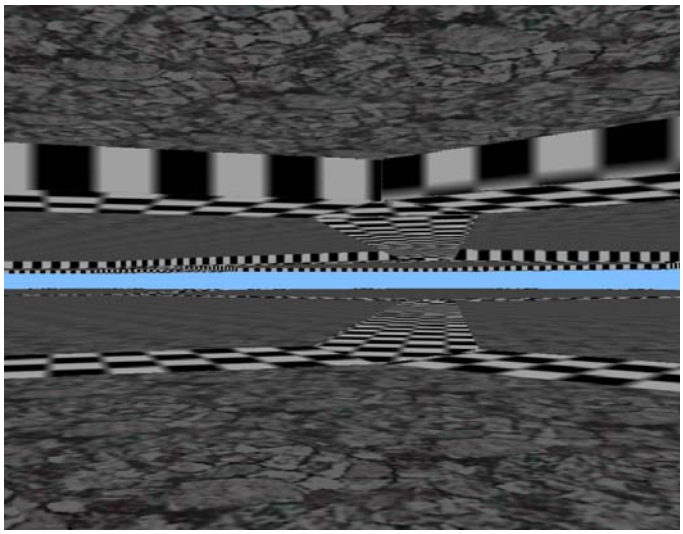


Fig. 41 Inside view of the second environment in Experiment 12-a; a Y-crossing is in viewing direction.

3.10.5 Discussion

Although the results seemed promising, the experiment was interrupted in favour of a more challenging experiment before we could achieve any final results. On the initiative of Christian Hölscher from the School of Biomedical Sciences, University of Ulster, Coleraine, we had the chance to perform a feasibility study on the combination of our setup with electrophysiological techniques in cooperation with Andre Fenton from SUNY, Downstate Medical Center, Brooklyn, NY, USA. For this study, animals were needed which could follow a ring shaped street pattern repeatedly, and since there was not enough time to train a new group of animals we decided to use the above mentioned animals. The results of this study are described in Experiment 15 (Chapter 3.14).

3.11 Experiment 12-b

3.11.1 Introduction

The second half of Experiment 12 was dedicated to the question if our animals interpreted the presented environments as a spatial object or if they could at least interact with them in a spatial way. The best known and mostly used test for spatial abilities in rats is the Morris-

water-maze-test (Morris et al., 1982). In this test, animals are forced to swim in a pool which is filled with opaque water and to search for a hidden platform that prevents them from drowning. To perform the spatial task of landmark navigation, they must be able to memorize the position of the hidden platform in relation to distal landmarks in viewing distance. In subsequent sessions, the animals learn the position of the platform in relation to the landmarks, which is confirmed by a decrease in duration and length of the animals' search path. Although this test was initially performed with rats (Morris et al., 1982), it proved its practicability for several different species, even humans (Astur et al., 1998). To prove the spatial abilities of the animals in our setup, we decided to adapt the Morris-water-maze-task to virtual reality. Another intention of this experiment was to create data which can be compared to real world data of other rat experiments as well as to virtual reality data from the experiments on humans.

3.11.2 Material and methods

The biggest difficulty in creating this task was the fact that we intended to keep the animals in a closed compartment without giving them force feedback. The environment in which the experiment took place consisted of a squared arena with an edge length of 20 m. The walls which enclosed this arena were 10 m high and were covered each with a different texture to make them distinguishable, so that they could serve as distal landmarks. Ground and roof plate were textured with the previously used gravel texture, the walls were textured with black and white stripes of different orientations (vertically, horizontally, diagonally right and diagonally left). As target area, we placed a vertically black and white striped column with a diameter of 5 m in the centre of the environment. The initial step to keep the animals inside the environment was punishment through reward deprivation. This means that the animals were allowed to freely explore the environment, where they received reward for every travelled meter under the centre column as long as they did not get closer than 50 cm to one of the walls. If they did so, however, the session was terminated and the animal was replaced by another one. The experiment was also terminated when the animals had spent more than two minutes under the column and had therefore received enough reward. After a short break, these animals got another chance to explore the arena under the same conditions as before. This procedure was repeated until the animals spent an overall time of approximately 10 -12 min in the environment. After 10 days of training, the animals showed a reliable tendency to stay inside the environment and had learned the concept of receiving reward underneath the column. The next change towards a Morris-water-maze task was the shift of the target column to a position with the coordinates 5/5, which means that it was now centred within one quadrant of the arena (Fig. 42 A/B). The animals still started in the centre of the arena at position 0/0, but now they had to learn to approach the column first. After this was achieved, we decreased the visual diameter of the target column in the following steps. Thus, we forced the animals to orient themselves more and more with the help of the distal landmarks. The diameter of the column was stepwise decreased by 50, 25, 12.5, 6.25 and 0 % of the original diameter. The diameter of the area in which the reward was given was kept constant during the complete experiment. Finally, we ended up in a situation which resembled the conditions of a Morris-waster-maze task quite well. The animals started in the middle of the arena and searched for an invisible area in which they received reward; to do so, they had to use the distal landmarks given by the surrounding walls. On a test day at the end of the experiment, the reward was removed, too, and the search pattern of the animals was analyzed.
To prevent the occurrence of fixed directions and of other influences from outside the setup, the virtual environment was rotated by a random multiple of 90° after every session.

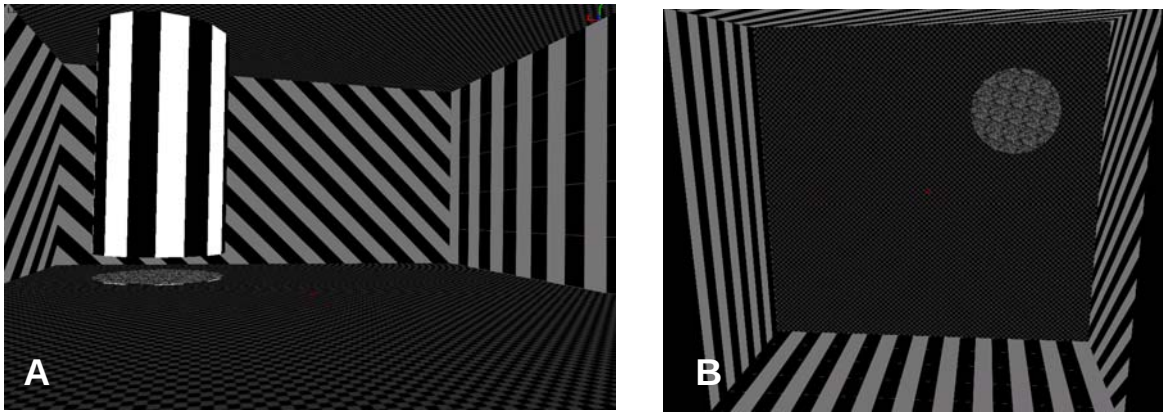


Fig. 42
A: Inside view of the environment in Experiment 12-b, with the target column at position 5/5 in the centre of the first quadrant. This image approximately renders the view height of the animals during the experiment.
B: Top view screenshot of the environment in Experiment 12-b, the target position and the different textures on each wall can be seen.

3.11.3 Results

The training revealed itself as medium effective to keep the animals inside the arena. Towards the end of the experiment, it occurred increasingly that the animals left the arena. Especially when the task became more difficult and the rewarding area was not as easy to find as in the beginning, the frustration level rose and their will to perform the task correctly fainted. Despite this decreasing motivation, the results of the test day clearly showed that the animals had learned the position of the rewarded area. Fig. 43A shows the sojourn time of the animals in the four quadrants of the arena. One can see that the animals preferably searched in the formally rewarded quadrant. The difference between the rewarded and the unrewarded quadrants is at least ** significant after t-testing with p = 0.02 Q1v.s.Q2, p = 0.001 Q1v.s. Q3, p = 0.003 Q1v.s. Q4. Fig. 43B shows the distance which the animals had covered in the four quadrants of the arena. The result is similar to the sojourn time and shows a preference for the formally rewarded quadrant. The data have also been t-tested at least ** significant with p = 0.05 Q1v.s.Q2, p = 0.007 Q1v.s. Q3, p = 0.01 Q1v.s. Q4. In Fig. 44, the distribution of the sojourn time over the quadrants for the individual animals is shown.

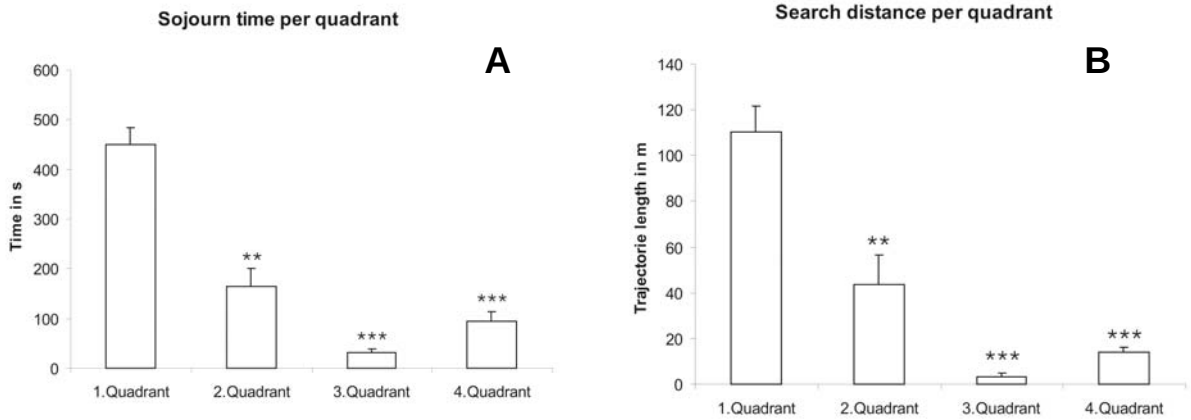


Fig. 43
A: This figure shows the sojourn time of the animals in the four quadrants of the arena.
B: The distance which the animals had covered in the four quadrants of the arena.

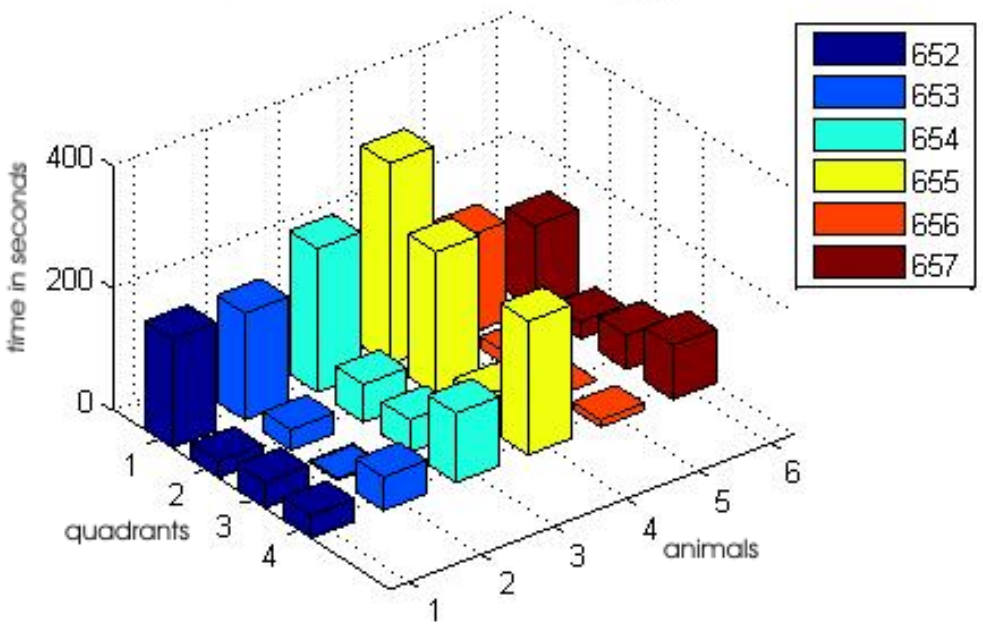


Fig. 44 The distribution of the sojourn time over the quadrants for the individual animals.

3.11.4 Discussion

Since this task has been repeated in Experiment 18, I decided to include the results of both experiments in one comprehensive discussion, which can be found in Chapter 3.17.4.

3.12 Experiment 13

Experiment 13 was the follow-up experiment of Experiment 11 (Chapter 3.9), in which the same environment and task were used as described before. The only difference between the two experiments was the colouring of the target columns. Whereas in Experiment 11 the columns differed in contrast, those in Experiment 13 had the same colour, but a different texture. One half of the columns was striped black and white from top right to bottom left, the other half from bottom right to top left (Fig. 45). The intention behind this experiment was to eliminate the different contrast which had influenced Experiment 11. We hoped that without this influence, the learning process would be accelerated. As in the experiment described before, only one type of the columns was rewarded and the animals should learn to discriminate between them.
Unfortunately, the animals did none of this. When after 18 days of experiment no sign of preference for the rewarded type of columns became visible and the hope for an accelerated learning was not fulfilled, we decided to stop the experiment although some answers remained open. Unfortunately, it remains unclear if the inability of the animals to discriminate

between the different types of columns was due to the features of the texture or if they would have needed more time to improve their performance.

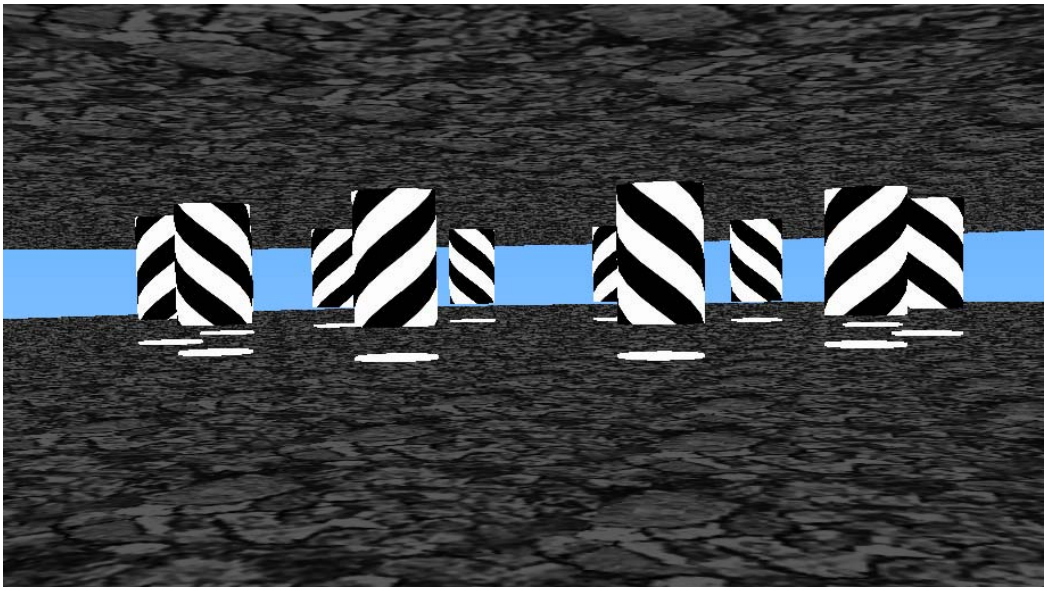


Fig. 45 Inside view screenshot of the environment in Experiment 13. The alternating texture with the diagonal stripes can be seen.

3.13 Experiment 14

Experiment 14 was a sequel of Experiment 11 and was intended to act as a pre-experiment for the Diploma thesis of Jan Regler (2006). Since this experiment represents only a preliminary test of the setup design, I decided not to present it here. The environment and the related task in it, which were subject of this part, have been exactly adopted for the initial experiment of Mr Regler's diploma thesis. For more detailed information, I would therefore like to refer to his work.

3.14 Experiment 15

3.14.1 Foreword to Experiment 15

Experiment 15 was a feasibility study for electrophysiological recordings in the rat V.R. setup. This project was only made possible by the cooperation of many colleagues. First of all, Hansjürgen Dahmen, who helped to expand the V.R. setup by installing the necessary technical features to perform electrophysiological recordings; secondly, Andre Fenton, who was the main source of knowledge about electrophysiological recordings and was kind enough to provide us with his two assistants; namely Eduard Kelemen, who did the recordings; and Hsin Yi Kao, who performed the surgeries to implant the electrodes; and finally Christian Hölscher, who initiated this cooperation and assisted us also with his knowledge about electrophysiological recordings. My own contribution to this project was the

training of the rats. Due to the fact that this experiment revealed some very interesting results and that these results have not been published so far, I decided to include the documentation of this experiment into my thesis although my contribution to it was only one part of many necessary steps assuring the success of this project. Especially the data presented in the result section were completely provided by Andre Fenton and Eduard Kelemen.

3.14.2 Introduction

As mentioned in Chapter 3.10, this experiment was the sequel of Experiment 12-a. The animals' pre-training of running along the streets made them ideal subjects for this experiment. With respect to the previous work of our contributors, the focus of our interest lay in hippocampal place cell recordings. The classical real world experiments all have one major constraint; they are limited by the dimension of their mazes. Almost all of the research in this field was performed in environments which are not much bigger than $2 * 2$ m. One can therefore imagine that there is a big interest in a method which allows expanding these borders. This need was one of the main motives for the construction of our rat V.R. setup. After the setup had proven its successful operation, the time had come to use its benefits for place cell recordings.
A crucial requisite for the analysis of place cell recordings is an explorative behaviour which leads the examined animals repeatedly to the same place in a given arena. In a real world arena with the dimensions of $1 * 1$ m, a rat can explore the complete arena multiple times within 10 to 20 min. With an extended arena size like the one we tried to use, this cannot be achieved within an acceptable experimental duration. Therefore we decided that the animals should not explore the complete arena at random. Instead, we used the pre-trained "street following" animals and gave them a ring shaped street to follow. This led the animals to cover big distances while visiting different areas of the arena, but assured that, while doing so, they repeatedly visited the same places on the "street ring".

3.14.2 Material and Methods

The recording equipment

The recording equipment was an Axona data acquisition system (Dacq) which included an amplifier, filter and digital signal processor module for a total of 16 channels. The Dacq system is designed for "tetrode" (twisted quadruple electrode) recording. The system consists of two main components: an industrial PC and a system unit which holds the plug-in amplifier cards.
Additionally, there were two preamplifier stages, of which one, the head stage, was directly mounted to the tetrode containing microdrives, the other one was in-between the cable and the system unit. One feature which was requested for the adaptation to the V.R. setup was a thin flexible connection cable that was able to pass trough the central canal of the rotational joint to which the animals were fixed (see Fig. 10). Surprisingly, we could achieve a usable signal to noise ratio without installing additional electrostatic shields although there are many sources (beamer, PCs, monitors) close to the recording device. The microdrives holding the tetrodes were self-manufactured and allowed us to move all four of them independently.

Animals and surgery

Six male Long Evans rats were treated, which weighted between 386 and 452 g at the time of surgery. The animals were numbered 646-651 and had an age of approximately 18 months. All treatments were performed under permission and according to the German animal protection law (TierSchG § 8Abs. 1). The tetrodes were implanted at position -4 mm on the anterior-posterior axis and 3 mm on the lateral axis relative to bregma. For anaesthesia, an 8:1 mixture of ketamine and xylasel was used. The animals had at least two weeks of recreational time until we resumed to the training.

The environment

In this experiment, we used an environment which consisted of a ground plate of the dimensions of 20 * 20 m. This plate was surrounded by 2 m high walls of the same texture as in Experiment 12-b. In the centre, a ring shaped street with a radius of 2.5 m was located. The street was 1 m wide, which leads to an outside diameter of 6 m and an inside diameter of 4 m for the ring. It was of the same design and texture as for the streets in Experiment 12-a, this means that is was also topped by blocks which floated over the street in a distance of 50 cm (Fig. 46A). In each corner of the surrounding environment, several objects were placed which should serve as landmarks. The objects were chosen to differ in number and shape in order to make them easily distinguishable. In the first corner, one cube was placed, the second corner contained three cones, two spheres were placed in the third corner and in the last corner, four columns were suspended from the ceiling. All objects were textured with a chequerboard pattern, except for the columns, which were vertically black and white striped. They also floated 50 cm above the ground and had size of 1 m; again the columns differed from the other objects since they had a diameter of 50 cm and a height of 1.5 m. Fig. 46B gives an impression of their location in relation to the street ring and the surrounding environment.

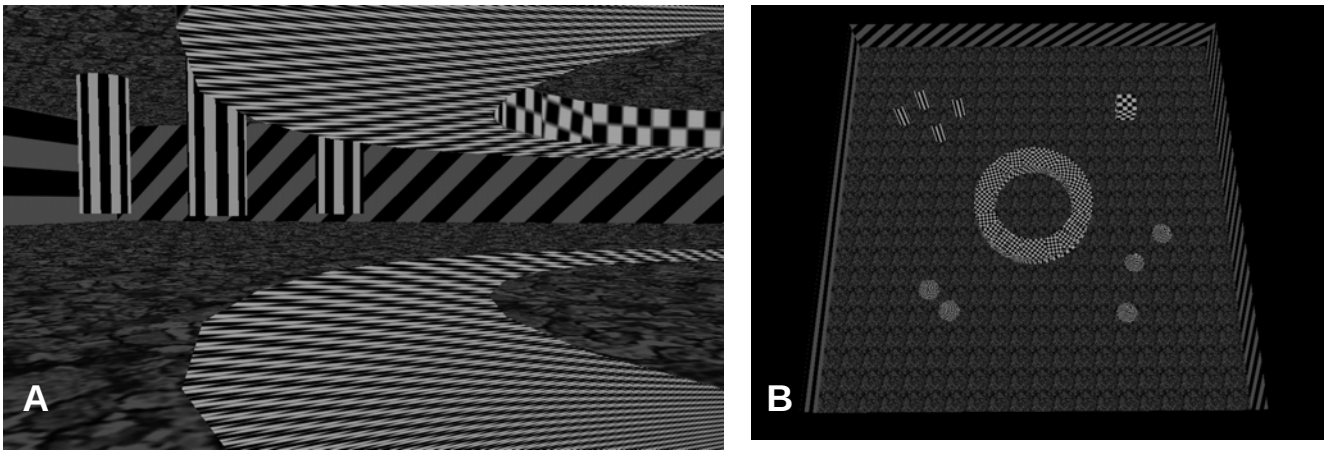


Fig. 46
A: Inside view of the environment in Experiment 15. This image approximately renders the view height of the animals during the experiment.
B: Top view screenshot of the environment in Experiment 15. The position of the street ring and the arrangement of the surrounding landmark objects can be seen.

The training

Although the animals underwent the pre-training described in Experiment 12-a (Chapter 3.10), the switch to a ring shaped street was easily realized. To support the adoption process, the reward was given at the beginning at a high spatial frequency (every 50 cm) and

independent from the direction. The frequency of the reward application was then reduced on the following days and, approximately half way trough the training session, only one running direction was rewarded. The training lasted 35 days until the surgery was performed and after the recreational time another 5 days of training were added. At that time, the animals showed a reliable behaviour of running along the ring, as it can be seen in Fig. 47. To prevent the animals from leaving the environment, a switch sequence like the one in Experiment 11 was triggered when the animals came closer that 50 cm to one of the outer walls. The occurrence of these events decreased to a negligible amount at the end of the training phase.

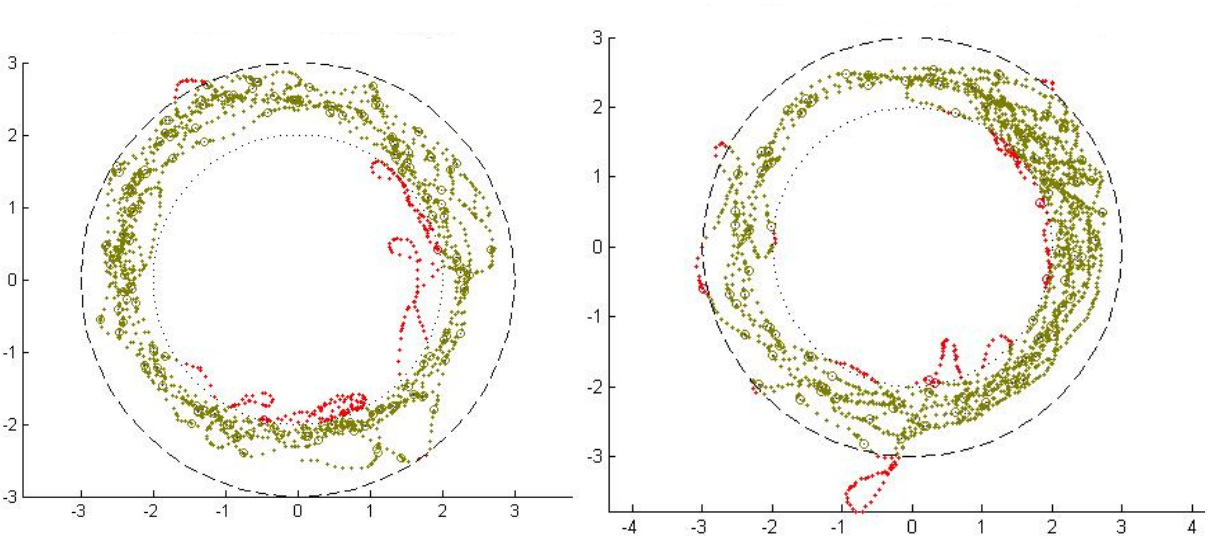


Fig. 47
Tow example trajectories from the training day before the surgery; the ring street is marked by the dashed line, the parts of the trajectory when the animals left the street are coloured red.

The program and the experimental procedure

The recording was performed by a programme which rewarded the animals every 2 m while they were running clockwise on the street. In contrast to all previous experiments, the duration of a single experimental session was extended to 15 min to ensure an adequate amount of complete rounds on the ring street. During the first days of the recording phase, a screening for cells was performed. After the animals had been transferred to the lab, they were connected for a short period to the recording setup while sitting in a small box. If no cell could be see on the oscilloscope, the tetrodes were lowered by 1/16 mm (1/4 rotation of the screw). This screening was performed twice a day. After the first spikes became visible, the screening for the respective tetrode was halted while it was continued for the remaining tetrodes. If a cell signal was obtainable, an experimental session as described above was performed during which cell activity was continuously recorded. If the motivation of the animal allowed it, a second and a third recording session followed. More than three sessions were not possible since, by the end of the third session, the motivation of the animals had dropped to a useless level. In the cases where no cell activity could be observed during the screening, just one simple training session (15 min) with no recording followed. Recordings were performed on nine successive days.

3.14.3 Results

Altogether, the hippocampal activity during running in a virtual environment could be recorded from 46 putative pyramidal cells in six recording sessions in two rats. In three rats, no cell could be found and in one of the rats cells could be found which did not have the characteristics of putative pyramidal cells. In Fig. 48, the average wave-forms, inter-spike interval histograms and autocorrelation plots for three simultaneously recorded putative pyramidal cells in the virtual reality setup are plotted. The peaks in the autocorrelation plots at 120 ms show that the cells' firing was modulated by an ongoing 7-8 Hz EEG oscillation (theta rhythm). The peaks at 5-7 ms in the inter-spike interval histograms show the tendency of cells to discharge in bursts with 5-7 ms intervals between subsequent action potentials. All of these characteristics look the same for hippocampal pyramidal cells recorded in standard environments.

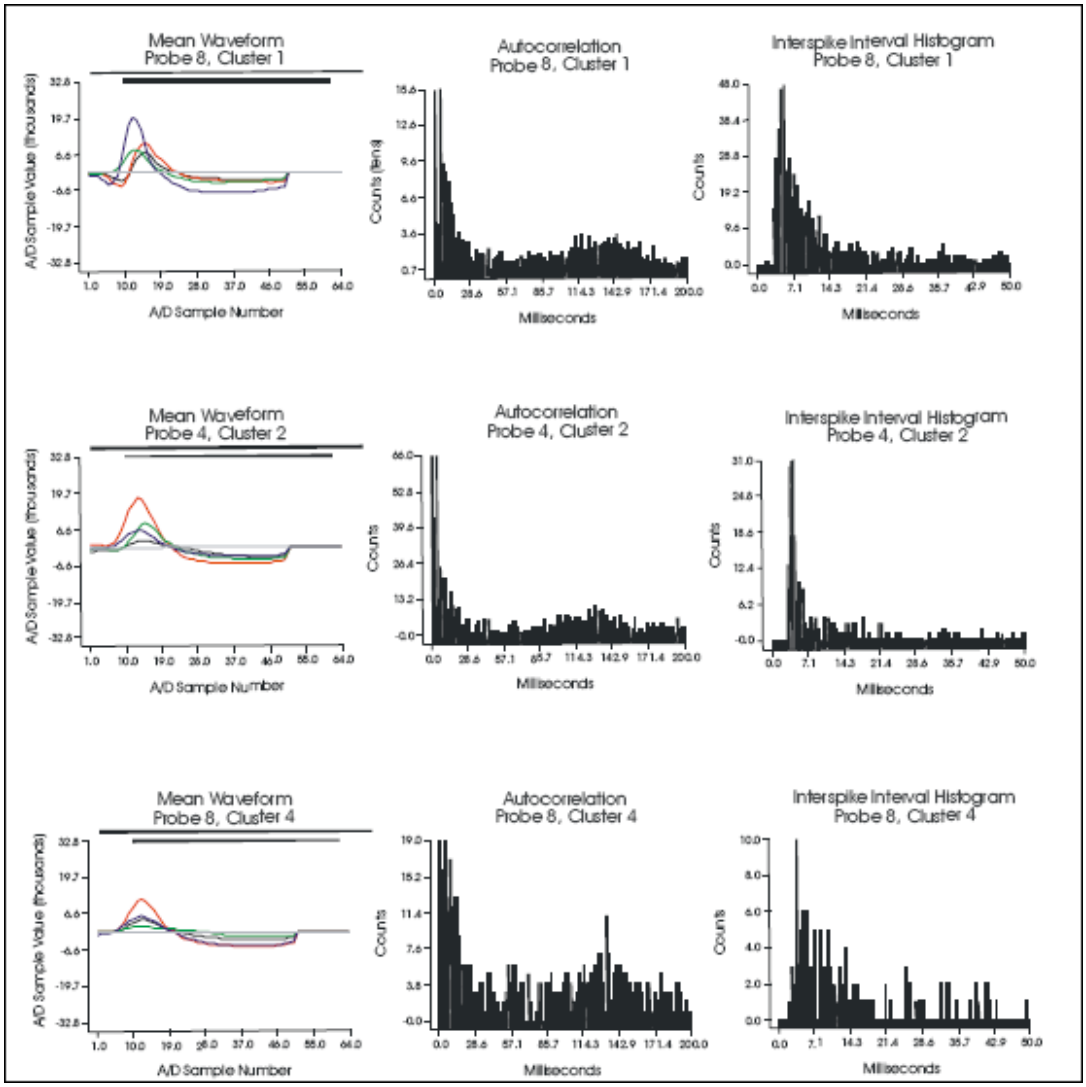


Fig. 48 Average wave-forms, inter-spike interval histograms and autocorrelation plots for three pyramidal cells in the virtual reality setup.

In Fig. 49, the spatially modulated activity of putative hippocampal pyramidal cells in virtual environment can be seen. Section A shows the activity of the same cell, which was consistent during three subsequent trials recorded on the same day. Section B displays the activity of a different, simultaneously recorded cell. In the top row, the locations of neuronal discharges are marked by red dots and are superimposed on the rat's trajectory marked in grey.

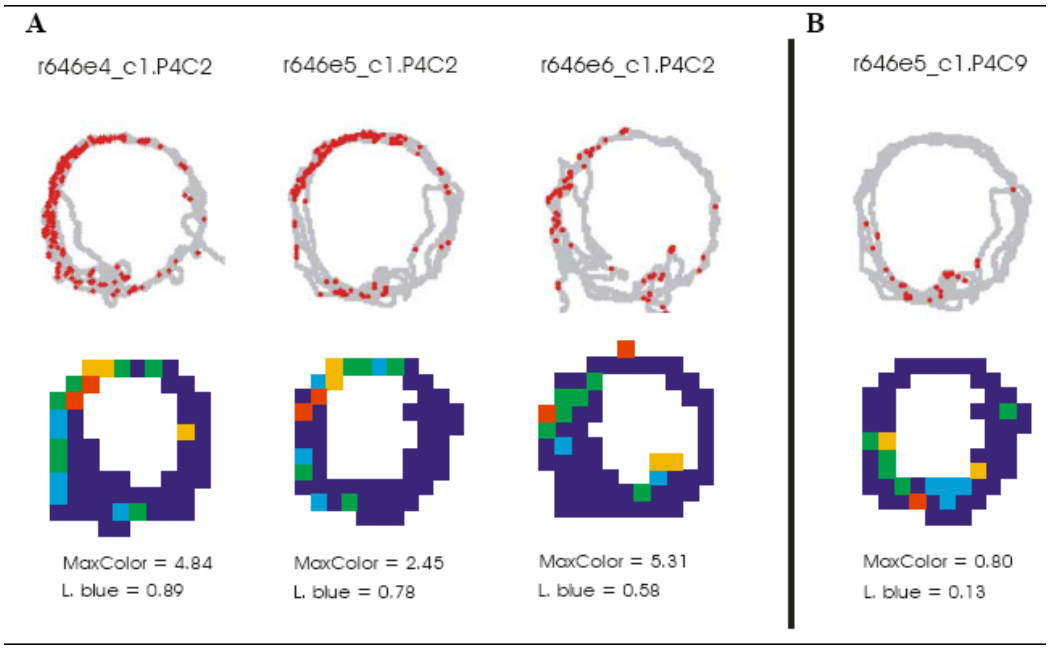


Fig. 49 Spatially modulated activity of putative hippocampal pyramidal cells in the virtual environment. A: Activity of the same cell was consistent during three subsequent trials recorded on the same day. B: Activity of a different, simultaneously recorded cell.

In the bottom row, the dark blue colour marks pixels with no firing and light blue, green, yellow and red colour marks pixels with increasingly higher average firing rates.
The plots in the left column of Fig. 50 show the distribution of firing of nine cells recorded during a single 15-minute session in a virtual environment. The rat ran 13 complete laps during this recording session. The trajectory of the rat is shown in grey; the places where a cell discharged are marked by red dots. Discharge of some cells (cells 1 - 6) appears to be restricted to certain locations of the environment.
To further analyze the spatial distribution of firing, the circular virtual track was divided into 18 sectors, each sector spanning 20°. For each of these sectors, the average firing rate of a cell was computed. The distribution of firing rates in different sectors is shown in the second column from the left. Obviously, if a particular cell has a spatially restricted firing, we can see a peak in this plot corresponding to the angle where the cell tends to fire most.
The spatial distribution of firing rates during two subsequent recording sessions is compared in the third column of Fig. 50. The blue line shows the same data as are shown in the second column, the thin red line shows the distribution of firing of the same cell recorded during a previous session. One can see that for most of the cells with spatially organized firing, the peaks of firing during the two subsequent sessions overlap. Correlation between the two distributions was computed and is shown in the right column of Fig. 50, along with its correlation coefficient. Note that the slope of the regression line is positive for all nine cells.

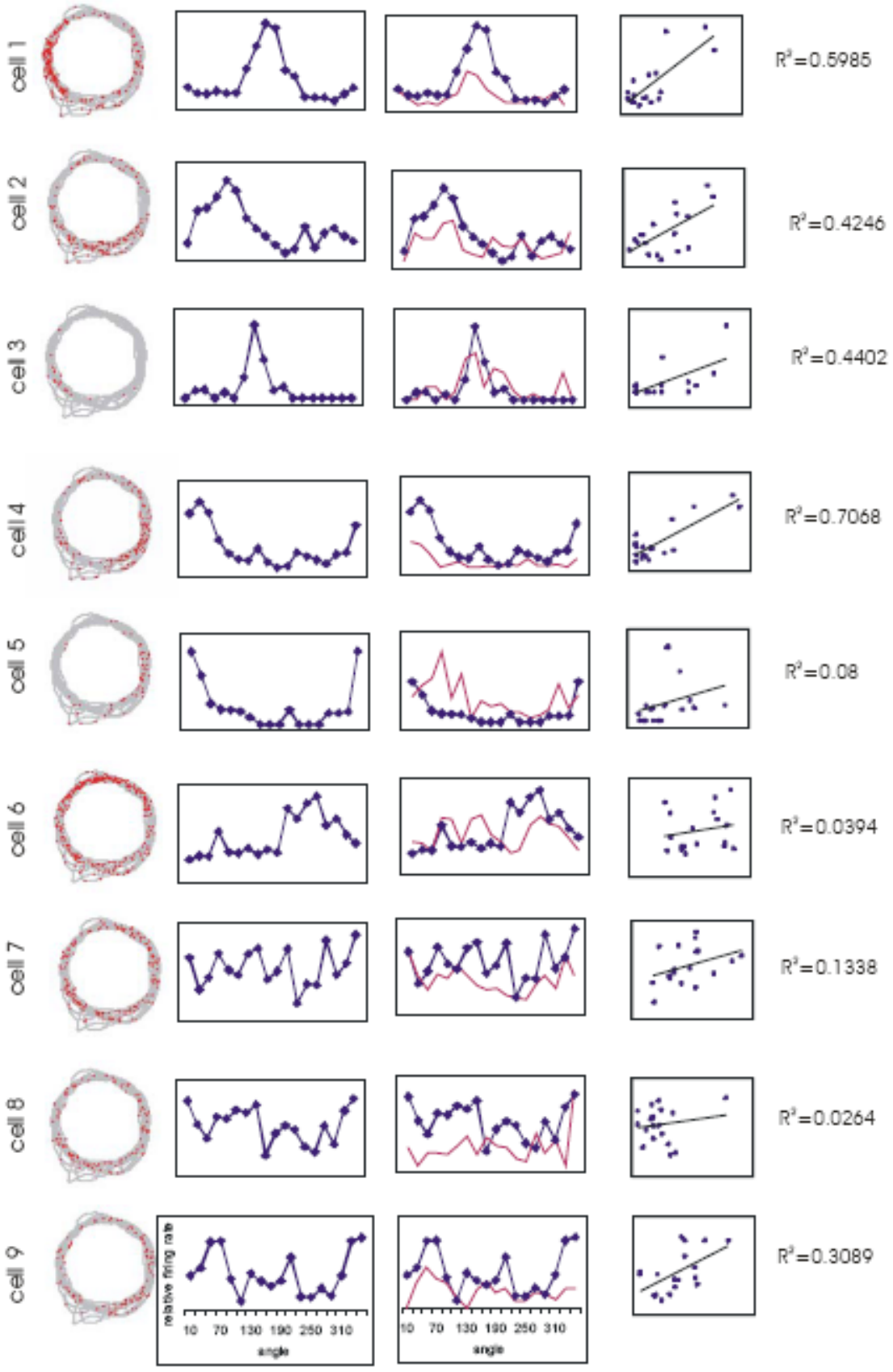


Fig. 50 The plots in the left column show the distribution of firing of nine cells recorded during a single 15-minute session (trajectory of the rat is shown in grey; places where a cell discharged are marked by red dots). In the second column, the distribution of firing rates in different sectors is shown. The spatial distribution of firing rates during two subsequent recording sessions is compared in the third column. The correlation between the two distributions was computed and is shown in the right column.

The same analysis was made for all 46 recorded cells. For each cell, the firing rate distributions during two subsequent sessions were compared and Pearson's correlation coefficient was computed. The distribution of correlation coefficients is shown by the blue bars in Fig. 51. This distribution appears to be bimodal. The two peaks of the distribution correspond to a correlation coefficient of 0 and 0.5.

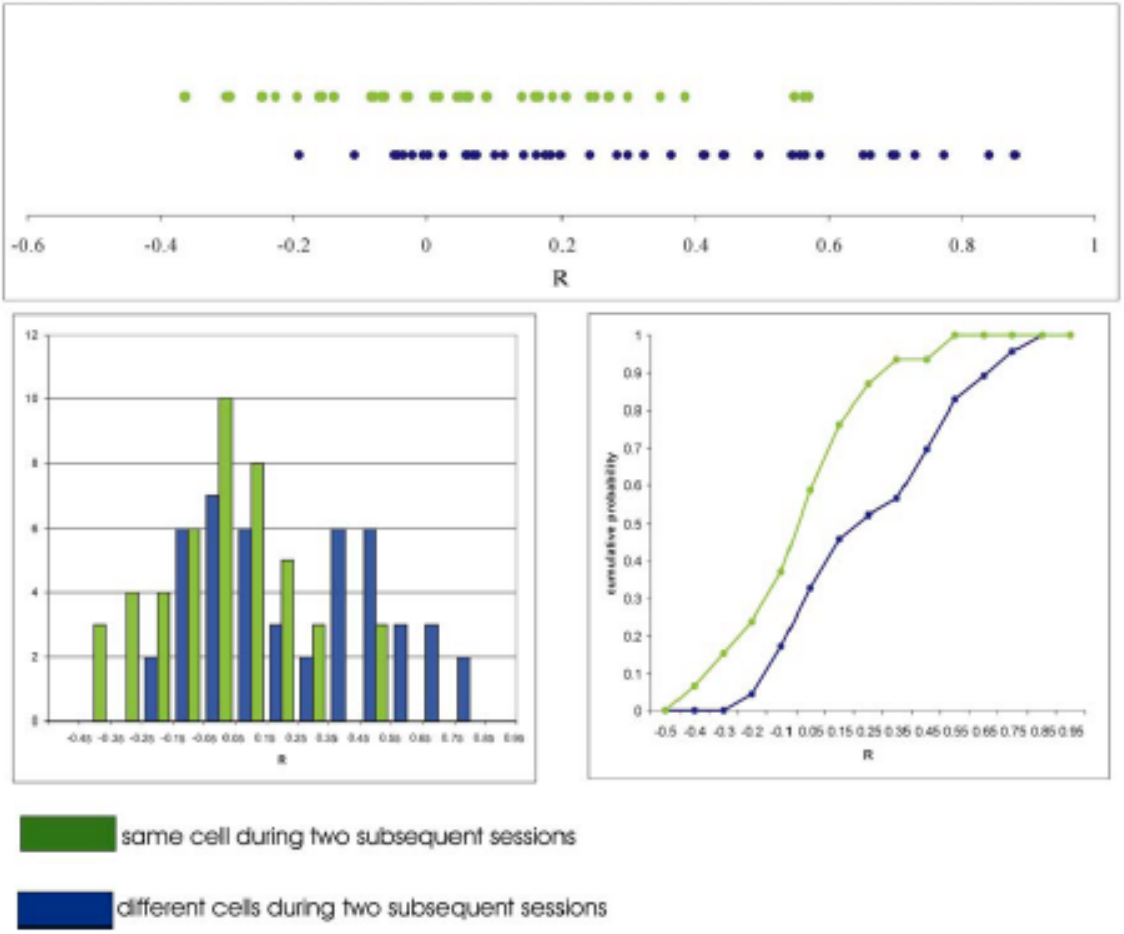


Fig. 51 The distribution of correlation coefficients for all 46 recorded cells (For details see text).

To approximate what correlation coefficients could be expected if the cells' firing during the two subsequent sessions was unrelated, we computed the correlations between the spatial distributions of random pairs of cells from two different recording sessions. The distribution of correlation coefficients between two random cell pairs is shown by the green bars in Fig. 51. As expected, the correlation coefficients of spatial firing of two different cells are distributed around 0. The two distributions were significantly different $t(90) = 4.57$, $p < 0.0001$.
The positions of the tetrodes were afterwards controlled by histological methods. In Fig. 52, an example image of a brain slice can be seen. The position of the tetrode ending in relation to the hippocampal layers is marked by the lesion in the tissue (red arrow).

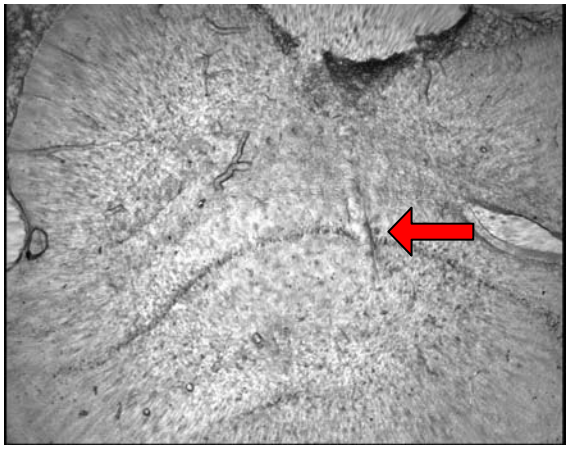


Fig. 52 Position of the lesion of the electrode at CA1 marked by the red arrow.

3.14.4 Discussion

As a feasibility study, which it was, Experiment 15 was a full success. We were not only able to overcome the technical obstacles we had expected when combining the two techniques of electrophysiology and rat V.R., but we also succeeded in recording cell activities which could be correlated with places in the virtual environment.

3.15 Experiment 16

3.15.1 Introduction

The design of Experiment 16 was inspired by a publication on triangle completion by Warren et al. (2001). For this task, subjects were led on a path which resembled two edges of a triangle. By integrating the distances and the angle of the two travelled edges, they generated a homing vector which led them back to the starting point. In the above mentioned publication, this task was performed in various virtual environments which differed in the amount of visual landmark information and therefore allowed issuing a statement about their role for the completion of this task.
After we had been able to prove that the animals use landmarks while exploring the virtual environments in Experiment 12-b (Chapter 3.11), the adoption of the triangle completion task to our setup seemed a very promising project. Our approach to realise this task intended, in a first step, to train the animals to follow triangle-shaped routes. We planned to mark these routes by the well known columns and, after these triangle routes had been learned successfully by the animals, to decrease the diameter of the third marker as in Experiment 12-b. Following this plan, we provided the animals with visual markers which guided them along the first two edges of the triangle and with an invisible home position. To reach this position, they had to perform the triangle completion task.

3.15.2 Material and methods

The environment which came into practice was an environment which should assure that the animals run reliably from one goal column to the next. Therefore, we created a repetitive ground and roof pattern which allowed the animals to run infinitely in every direction, as described in Experiment 1. In contrast to all previous experiments, the columns were placed dynamically. When the experiment started, the animals found themselves under a column and received a reward. A second column was also present from the start. The position of the column was calculated from the running direction of the animal, to which a random angle of +/- 30° was added at a distance of 2 m relative to the centre of the two columns. If the animal reached the second column, a reward was delivered, too, and the first column disappeared; simultaneously to this, a new column was placed at a position which was calculated as described for the second column. To ease the beginning of this training, the new columns were placed within a fairly narrow margin relative to the running direction of the animals. During the progression of the training, this margin was stepwise increased until, finally, the columns were placed in a completely random direction which varied over 360°. In the case that an animal did not reach the new column, a function was included which placed a new

column according to the above mentioned calculations if the animals moved more than 3 m away from any of the columns.

3.15.3 Results and discussion

The experiment failed already during the training period. After the first couple of days, when we started to increase the angle variation in which the new column should be placed, it became obvious that more and more columns were ignored. A close look on the trajectories revealed that columns which were placed beyond an area of +/- 90° around the running direction were almost never visited. As soon as this behaviour became apparent, we tried to interfere by introducing some corrections of the programme. One correction was a smaller step size by which we increased the angular variation. Additionally, the distance at which a new column was placed was increased from 3 m to 5 m. During the experiment, we had the subjective impression that, instead of making a sharp turn, the animals preferred to run a longer distance in the hope that the next column might be placed in a more acceptable angle. We hoped to weaken this preference by increasing the distance and therefore the costs to reach a new column. Unfortunately, all of these attempts could not change the effects of the above mentioned behaviour. In order to perform a triangle completion, however, turning angles of more that 90° were inevitable. At this point, we saw no possibility to force the animals to perform complete triangle runs; therefore we decided to terminate the experiment.

3.16 Experiment 17

Experiment 17 was not designed to answer a specific question, it rather should test the feasibility of a newly implemented part of the setup itself. This additional feature was a punishing device. The rewarding device, as it is described in Chapter 2.4, was modified by adding a second tube which was identical to the reward tube. Instead of sugar solution, we delivered short puffs of compressed air through this one. This should be a sort of punishment which is not so intense that the animals completely refuse to interact with the setup, but by varying the pressure and the frequency of the puffs we hoped to make it uncomfortable enough to guide the animals into or away of certain directions. To test the efficiency of this supplementary element, we created an environment that consisted only of a ground and a ceiling plane which were coloured black and had a diameter of 100 m, which was sufficient to prevent the animals from leaving the environment. On the ground plane, white circles with a diameter of 5 m were arranged on a squared grid with an edge length of 10 m, which resulted in a distance of 5 m between the circles (Fig. 53). The animals could freely explore this environment, but as soon as they entered the black areas the punishing device started to deliver air puffs with a duration of 100 ms and a pressure of 2 bar every 5 sec. As long as the animals remained inside the white areas, reward was delivered for every 2 m of travelled distance to avoid that the animals simply kept sitting still. In the beginning, the punishing device proved to be very effective, but after several sessions a certain habituation was observable. While the first encounter with the punishing pulses caused the animals to turn around or back up instantaneously, it later did not deter the animals from entering the black areas although they seemed to be still unpleased when a puff was delivered. Another problem which occurred was that some of the animals began to develop a keratitis after some days of the experiment. After the animals had recreated from this, we reduced the pressure to 1 bar

and, since then, this problem did not show up again. However, with the reduced pressure the habituation to the pulses rapidly took place.
Finally, we can say that the punishing device is a helpful supplement to the setup, which allows us to indicate the animals' go and no-go areas; however, its intensity and reliability are not as good as we wished it to be, especially when the effect weakens after a certain time.

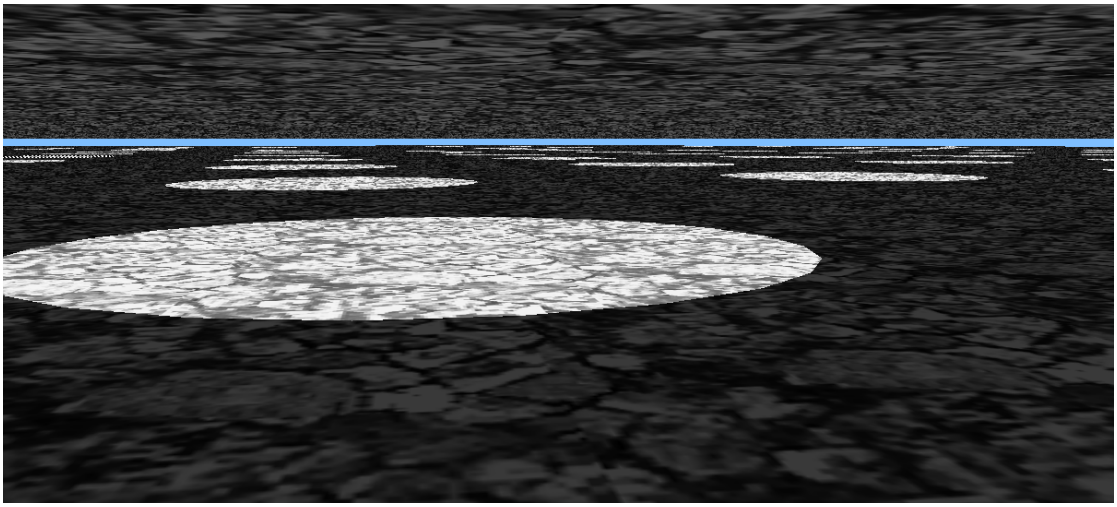


Fig. 53 Inside view of the environment in Experiment 17. Unpunished white areas and the punished surround can clearly be distinguished. This image approximately renders the view height of the animals during the experiment.

3.17 Experiment 18

3.17.1 Introduction

Experiment 18 is a rerun of Experiment 12-b, in which we tried to simulate a Morris-Watermaze-task. Although we received some promising results in Experiment 12-b, there always remained an unsatisfying feature in this experiment which overshadowed its outcome. We were not able to present absolutely convincing results because the number of subjects (6 animals) was slightly too small to yield a representative cross section over the individual variability. Another new feature which inspired us to a remake of Experiment 12-b was the now available punishing device which was introduced in Experiment 17. In Experiment 12-b, a lot of the training time was expended to train the animals not to leave the arena. By using the punishing device in addition to other methods, for example different sounds, we intended to shorten the training procedure significantly and to possibly get rid of the necessity to split the experiments in several sub segments. The second part of the experiment was then also extended by a new experimental sequence which allowed us to differentiate several possible influences on the outcome. After a series of unsuccessful attempts, we decided to use a procedure which had already proofed its feasibility and tried to substantiate the data we already had from our water maze simulation to prove that the animals are able to perform spatial tasks in the virtual environment.

3.17.2 Material and methods

The animals which came to use for this experiment were animals which were introduced in the experiments of Jan Regler (Nr. 91-104) and were therefore not naive. During the intermission between Part 1 and 2 of Experiment 18, those four of the animals which showed the worst performances were excluded from the following experiment, so that the final results were derived from the performances of animals 91,92,93,94,95,97,98,99,101,103. The animals participated in the experiment in order of their numbering, but this sequence was reversed every second day.

As mentioned above, the experiment was split into two subsections which differed basically in the introduction of a new experimental procedure in Part 2. Part 1 was an exact copy of the experiment which is described in Experiment 12-b, except for one difference. This was the punishing device which started to deliver air puffs every 5 sec after the animals had left the boundaries of the arena and, in contrast to Experiment 12-b, the experiment was continued until an overall experimental time of 600 sec (10 min) was reached. This also included that the animals did not perform the experiment repeatedly as in Experiment 12-b, instead, they participated just once a day for the above mentioned period of ten minutes. The main part of the experiment remained the same, i.e. we trained the animals to run towards a target area which was marked by a column. This column was decreased in diameter later on to guide the attention of the animals towards the distal landmarks which should enable them to find the area without the column by using landmark navigation (see also Experiment 12-b).

However, without the restart of the experiment, the rotation of the environment, which should prevent the development of fixed directions, took place only once a day for every animal. This and the above mentioned possibility to differentiate between various influences were the triggers to introduce the new experimental sequence.

The two factors of interest were the orientations of the environment (of which we had four) and a possible influence of the starting point. Therefore we introduced three starting points which were equally distanced from the virtual position of the target column in the diagonally opposite quadrant. These points were chosen in such a way that we kept the “old” starting point in the centre of the arena and added two other points which were located in the quadrants neighbouring the target quadrant. To make these factors distinguishable, we put them in a sequence in which every factor was once combined with the others, which ended up in twelve possible combinations. This meant, on the other hand, that there was not much time left for each trial if we wanted our animals to perform the task under all twelve conditions within an endurable time span. We decided that one minute for each trial should be enough for a trained rat to run from a starting point to the target. To prevent the possibility of overburdening the animals, we began the training with half of the sequence and two minutes for each trial and then decreased the duration stepwise until one minute was reached. To place the animals at the different starting points, a shift mechanism (see Chapter 3.9) had to be introduced. In a nutshell, the experiment was running like this: first the animal started at one of the three starting points, explored the environment and received a reward after it had entered the rewarded area. Secondly, when the first minute had expired, the animal was transferred to another starting point after the environment had turned white for three seconds, as described in Experiment 11. Simultaneously, the testing environment was rotated by a multiple of 90°. This was repeated until the animals had encountered all twelve combinations of place and orientation. The sequence of places and orientations was fix and not random, which would have made it difficult to compare them. However, to prevent that the animals learned this sequence, it was reversed every second day.

At the final step of the experiment, when no visible column was left, experiments were performed on four days under these conditions to cover all possible combinations of the above mentioned sequences. Since we could observe a performance drop between test day one and

test day two, an intermissive training day was introduced on which a visible target was presented again to raise the motivation of the animals. In contrast to Experiment 12-b, where no reward was given on the test days, we decided to continue rewarding in Experiment 18 for two reasons. The first reason was the fact that we needed four test days and tried to prevent a motivational loss towards the later test days. The second reason was the short duration of the trials which caused us to shift our attention from the search time, which was relevant in Experiment 12-b and would have been influenced by the reward, towards the approach time, which was influenced less by the reward. The time span the animals can spend in the rewarded area, which can be prolonged by giving reward, is rather short compared to the time they need to get there.

3.17.3 Results

In Fig. 54A, the number of target hits is shown. In this case, we counted the number of trials in which the animals managed to enter the rewarded area at least once. It can be seen that in every second trial (50.62 %) the animals were able to enter the target area. This is remarkable in terms of the fact that the target area covers only 4.908 % of the experimental area. In Fig. 54B, the same value for the single test days is displayed. The pattern that performance values are best for the first day and worst for the third day appeared repeatedly throughout the following evaluation.

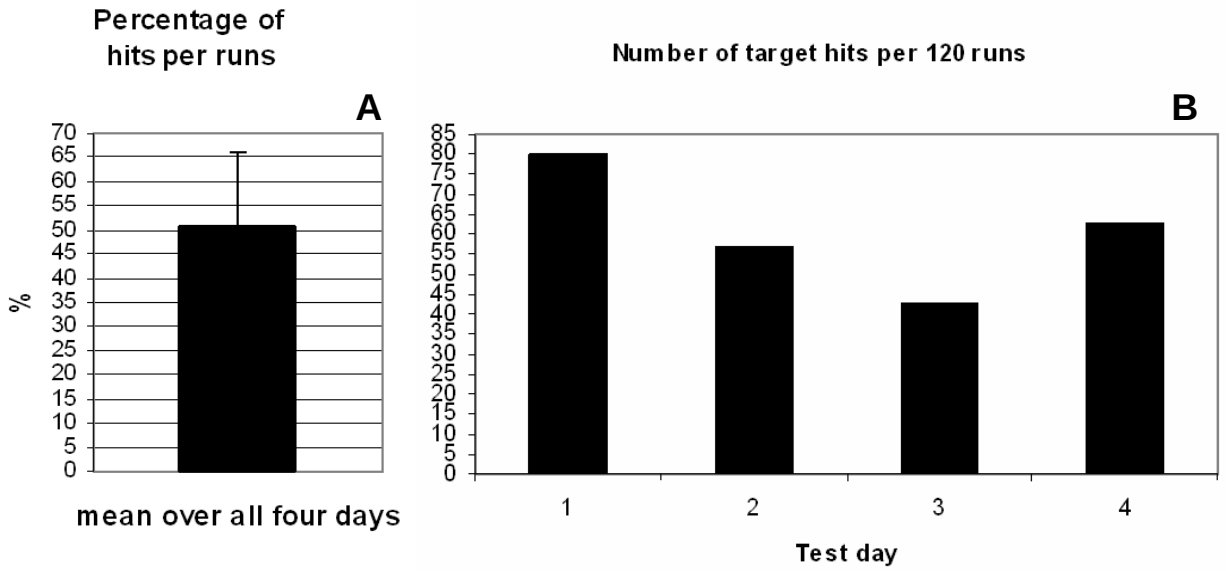


Fig. 54
A: In this plot, the number of target hits is displayed.
B: The number of target hits for the single test days is shown.

A further feature which was analysed is the distribution of the animals' trajectories over the test arena. Fig. 55 displays a density distribution plot over all animals and test days; the red circle marks the rewarded area. A clear peak can be seen within the reward area and three sub-peaks at the starting points, which shows us that the animals mainly travelled from a starting point towards the target area. For another analysis of this distribution, the arena was cut in four quadrants and time and distance which the animals spent within those quadrants were analysed separately (quadrant one is the target quadrant). Fig. 56A shows the time spent in the four quadrants of the arena over all four days and all ten animals. A clear difference between the first and the other three quadrants can be seen. This also becomes clear when the data are plotted separately for the four test days (Fig.56B).

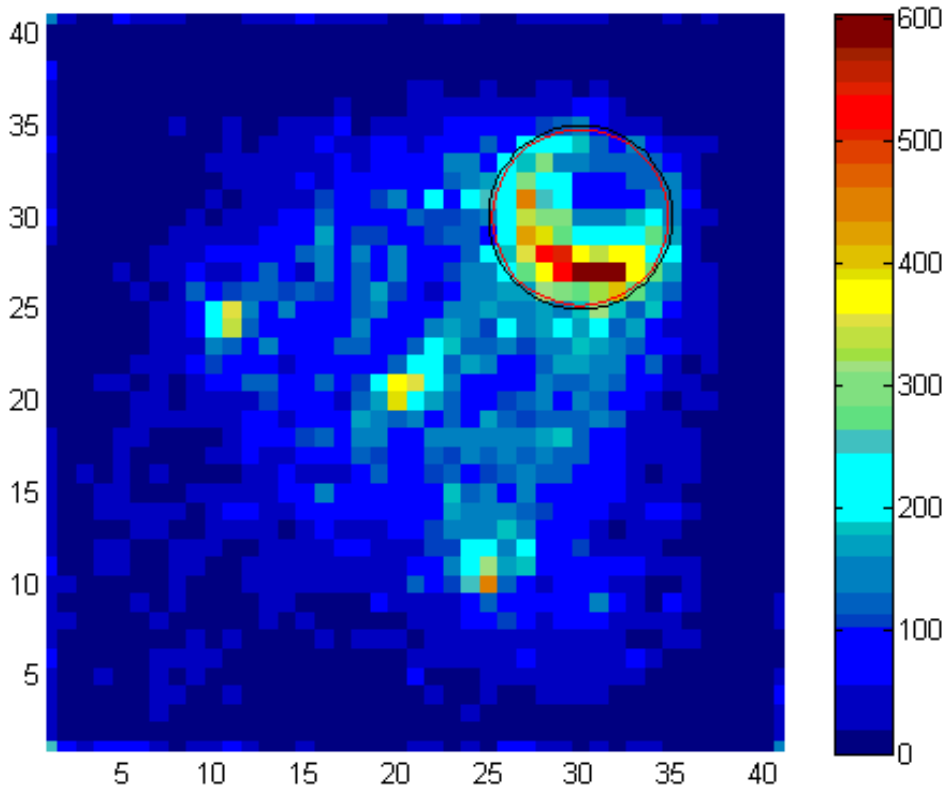


Fig. 55 This image displays a density distribution plot over all animals and test days; the red circle marks the position of the target area.

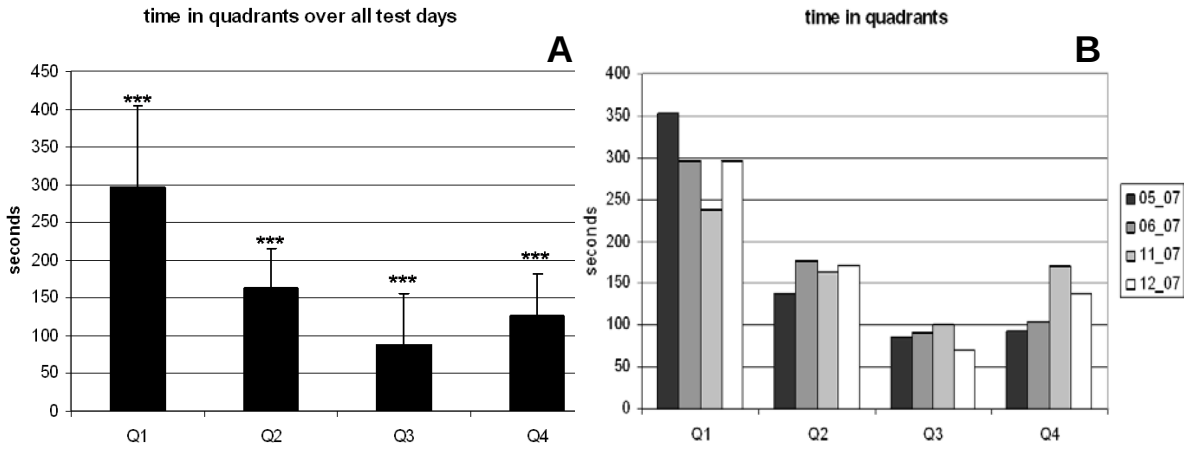


Fig. 56
A: The time spent in the four quadrants of the arena over all four days and all ten animals.
B: The time spent in the four quadrants of the arena over all ten animals separately for the four test days.

An ANOVA over the quadrants revealed a significant difference between them. ($F_{Quadrant}$ (3, 1920) = 67.68; $p < 0.001$). There was no interaction with the starting point. ($F_{Quadrant * starting\ point}$ (6, 1920) = 13.35; $p < 0.001$). An interaction with the orientation of the environment could not be found either. ($F_{quadrant * orient}$ (9, 1920) = 10.79; $p < 0.001$). Fig. 57A shows the distance covered in the four quadrants of the arena over all four days and all ten animals. Fig. 57B again displays the data separately for the four test days. The results of this evaluation are comparable to the time evaluation and show no further effects.

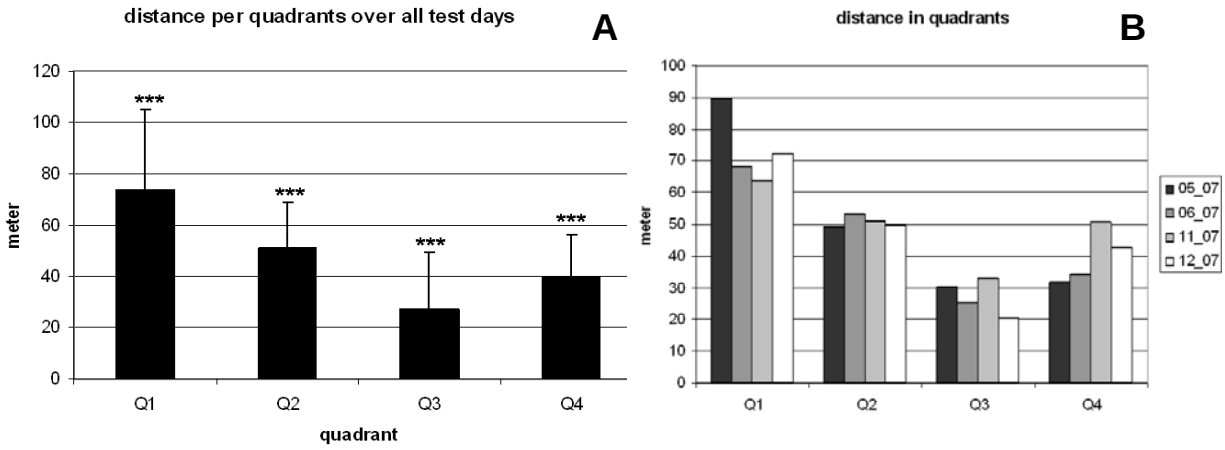


Fig. 57
A: The distance covered in the four quadrants of the arena over all four days and all ten animals.
B: The distance covered in the four quadrants of the arena over all ten animals separately for the four test days.

Fig. 58 shows the dispersion of the walking errors, which were calculated as actual working angle minus the angle towards the centre of the target. As we expected, a clear peak into the target direction (0°) is observable.

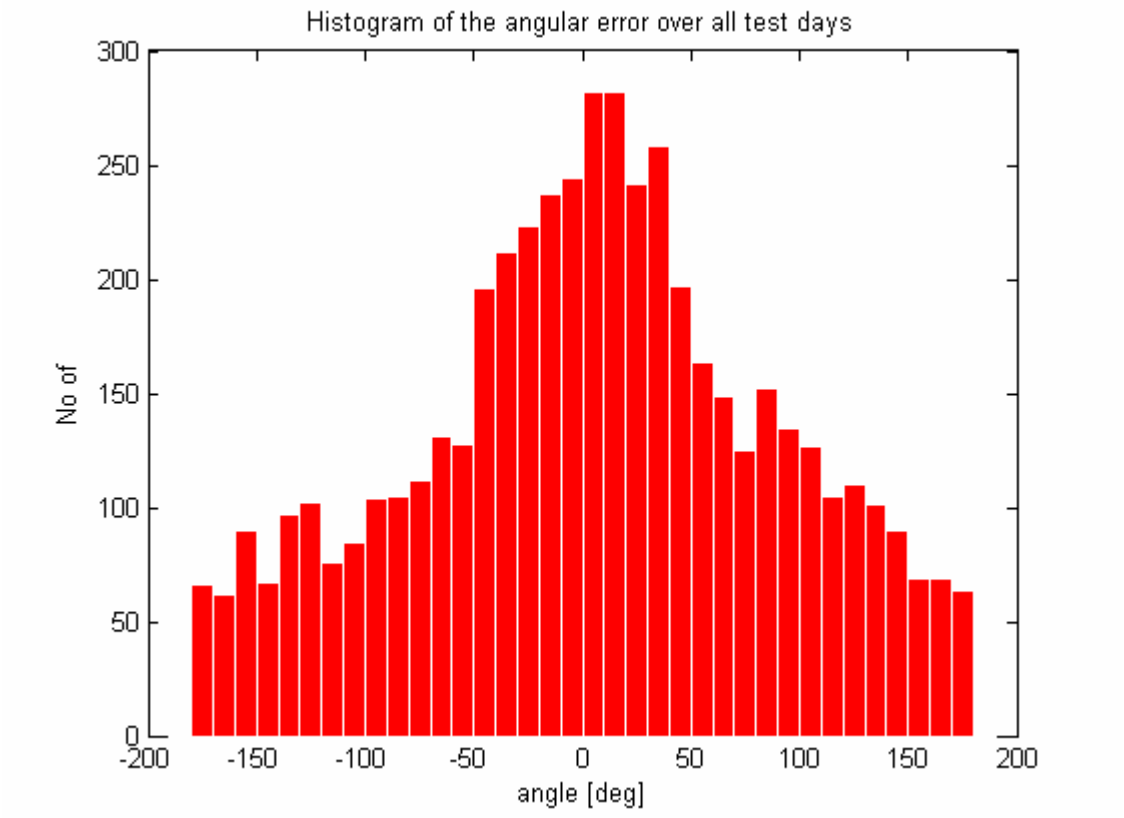


Fig. 58 This image displays a histogram of the dispersion of the walking errors over all animals and test days, which shows a clear peak at 0°. The zero direction represents the direction towards the target.

In Fig. 59, all trajectories of the single trials over all days are plotted together in coordinates relative to the fix lab environment (Fig. 59A) and in coordinates relative to the rotated virtual environment (Fig. 59B). The figures show that in V.R. coordinates, a clear directionality is observable, whereas it is not for the lab coordinates, which proves the influence of the virtual environment. Unfortunately, we could find two animals (95,101) which preferred to run into

fixed lab directions, as it occurred in experiments 3,4,5,6,9,10 (Chapters 3.4; 3.7; 3.8). The trajectory plots of these animals reveal this behaviour clearly although all trajectories go into the same direction when plotted in lab coordinates (Fig. 60).

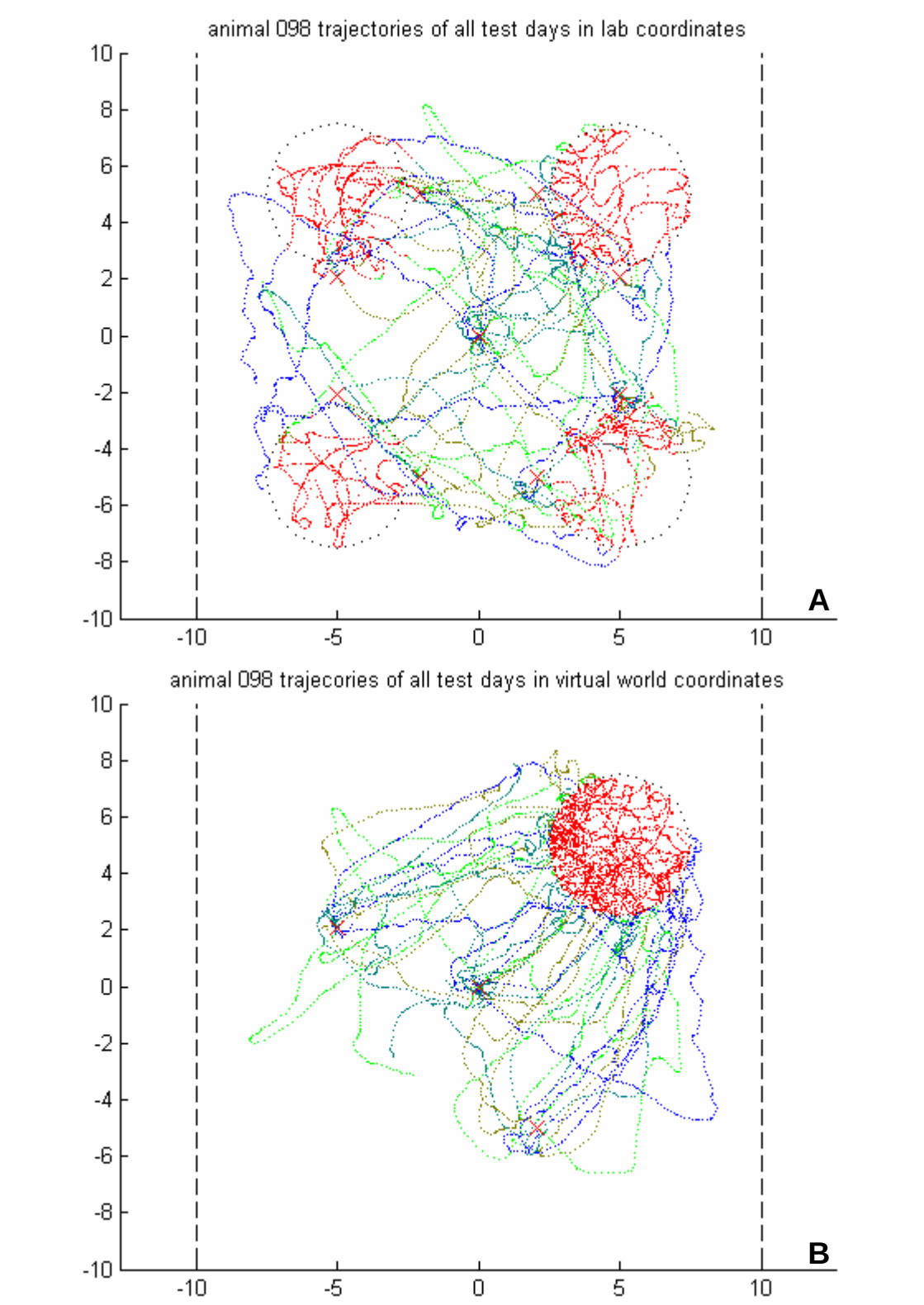


Fig. 59 Trajectory plot of the single trials from one example animal (098) over all days. A: displays these trajectories in coordinates relative to the fix lab environment; the target area is marked by the black dotted line and appears at four different positions. B: displays the equivalent trajectories in coordinates relative to the virtual environment; the target area is marked by the black dotted line and appears at the same position

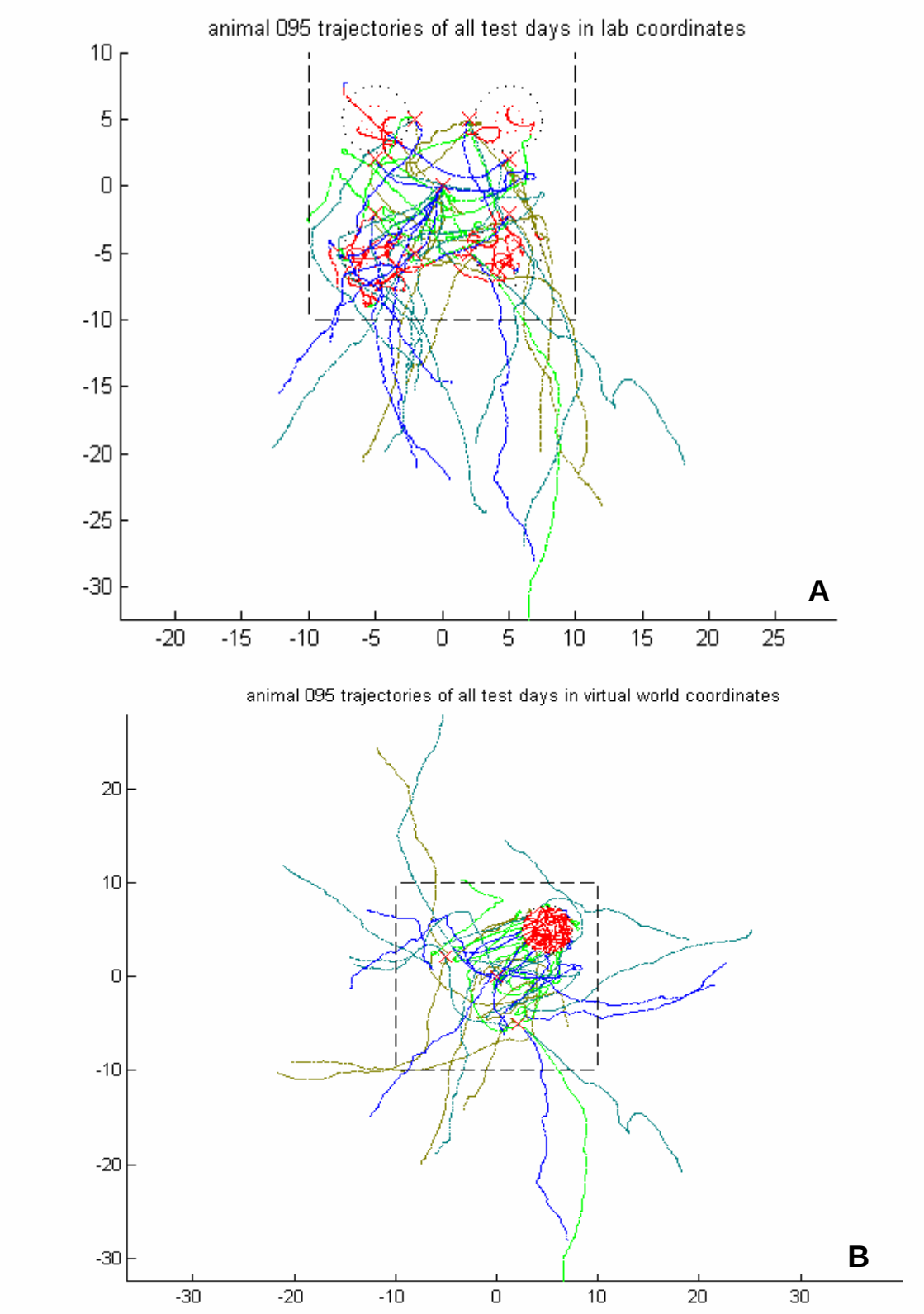


Fig. 60 Same type of plot as in Fig. 59 for animal 095.

An additional evaluation of the above described performance values was again carried out, leaving out the two animals which followed fixed directions; however, this caused only a minor amelioration of the performance. For this reason, and to keep the n at least at the number of ten, they were not excluded from the final evaluation. All error bars display the standard deviation.

3.17.4 Discussion

The major goal – to give evidence of the animals' ability to perform spatial tasks within a virtual environment – could be achieved; there are, however, many issues left to be discussed.
The angle dispersion shows a directed behaviour towards the target and the density distribution plot shows that this direction concentrates in the target area. The quadrant analysis on the other side makes clear that this behaviour is significantly different from random, which is also an affirmation of the data derived in Experiment 12-b. With this in mind, we can only conclude that the animals used the distal landmarks (the walls) to approach the target area and therefore performed successful landmark navigation within a virtual environment. Surprisingly, the performance of the animals which have been reported to follow a fixed direction was not as bad as one might expect, but this is due to the fact that they were also willing to deviate from their direction by a certain degree to receive a reward. This led to a behaviour which enabled them to occasionally make some target hits, but not as much as the remaining eight others.
The biggest difference between virtual and real world experiments is the enormous amount of training time. An animal in a real water maze is able to find the hidden platform quite reliably after five to ten trials. Part one of our experiment lasted 39 days, including the test days, and after this the performance on the task was disappointing. Another 38 days of training had to be added to achieve the presented performance. Compared to the duration of Experiment 12-b with 47 days of training, we can argue that the duration of Experiment 18 might also be due to the fact that we did not use naive animals and that they probably had to unlearn the old task first. An additional factor might be the age of the animals of one and a half years at the beginning of the experiment. As reported in Gallagher et al. (1993), the learning performance of animals of that age (or older) is significantly lower than in young rats. One can also argue that when trying to escape the water tank, the animals are more motivated than by just receiving sugar solution. However, beside all those restraints, we still have to deal with five to ten times longer learning phases than in a real experiment. This is a problem which also appeared in other V.R experiments, for example in Experiment 11 (Chapter 3.9).
The next difference between a real and our virtual water maze are the dimensions. A normal pool for water maze experiments has a size of 1.5 - 2 m in diameter, whereas our arena was 20 $*$ 20 m in size. But a more relevant item is the ratio between the arena area and the target area. In our environment, this ration is 20:1; in contrast to this, the ratio in the original Morris experiment (Morris et al., 1982) was 273.5:1. When taking into account that in a real world experiment the animals almost always find the target after some training, which happened only during 50 % of the trials in the virtual environment, one has to accept that the accuracy in virtual environments is rather poor.
In summary, we received the impression that with this task, the top level of complexity for our setup was reached. It is doubtable whether a longer training would have improved the results which were presented.

4 Discussion

The experimental section has provided an overview of the successful and unsuccessful attempts which have been carried out using our rat V.R. setup. Here I would like to discuss the error sources which interrupted some of the experiments; furthermore, I want to talk about the promising aspects of the setup and how they can be used for further investigations.

4.1 The "fixed direction" problem

At the beginning of our investigation, we did not control the movement directions of the animals against the real world directions. Only after the failure of Experiments 3-6 did we realize that the occurrence of this behaviour might have a crucial influence on the outcome of our experiments. One point that emerges from several experiments in which we encountered this problem is the fact that if no special recursion is taken to prevent it, the problem emerges in all animals, independent from the task, after a certain duration of training (approximately 5-10 days). Due to the fact that the animals preferred different directions, we can exclude that the development of this preference was induced by attracting or distracting factors from outside the setup, such as an uncomfortable sound source etc. Yet, there are several elements in the laboratory which are still perceivable within the environment. The projection system right above the animals is always visible and can give a cue about the lab directions. There is also a gap between the screen and the running ball through which the animals can see a stripe of the lab floor. Finally, there are many sources of sound and odour cues which we could not control for. To finally identify the source of this behaviour, we would have to modify the setup in a way which would allow us to rotate it. With such a setup, we would be able to revolve the possible sources independently and therefore isolate the relevant cue. But as this modification has not been executed so far, the question remains still open.
Another interesting feature of this behaviour is its persistence. In experiments 10 and 16 (Chapters 3.8; 3.15), we were by no means able to distract the animals more than 90° from their fixed direction. However, when we prevented the occurrence of the directions by rotating the environment from the beginning, like in Experiments 12-b (Chapter 3.11) and 18 (Chapter 3.17), we could show that it is possible to suppress the development of fixed directions. In our eyes, this underlines the argument that the cues which induced it were not of an attractive or distracting kind. My hypothesis concerning this behaviour is that if the task does not require greater deviations from a running direction, the animals stick to the direction which they can perceive from the above mentioned cues and if, moreover, they receive a reward by following this strategy, the direction becomes more and more imprinted. If the animals were, however, distracted from the beginning, they had to learn that the real world cues do not help them to reach their goal and that they have to ignore them.
Unfortunately, this hypothesis does not explain why the behaviour is so persistent even if it leads to a complete miss of reward (see next subchapter). Another explanation might be based on the moving behaviour of the animals on the ball. Most animals run short distances at a high velocity (about one meter), then stop, do some course corrections at a very low velocity, add another high sped phase and so on. Such run and stop sequences are not unusual in the normal movement behaviour of the rat. However, to change its moving direction when running on the sphere, the rat has to change the angular momentum of the sphere. The higher the velocity of the animal is, the bigger the angular momentum becomes, and therefore also the force which needs to be applied to change the running direction. This might lead the animals to run

straight ahead and, when doing so, to choose a fix element towards which they can guide their movements. To finally control for this effect, we would have to isolate the elements at which the animals orient themselves, regardless if they are elements inside the setup (the projection apparatus, the fixation apparatus or the sphere itself), or outside the setup (sounds, odours or what can be seen trough the gap between screen and sphere). This isolation can only be achieved if the whole setup or its elements (sphere, screen and projector) are rotated independently. As long as these experiments have not been performed, no definite statement about the source of the fixed directions can be made.

4.2 Misdirected motivation

Closely related to the fixed direction problem is the problem of motivational changes. Like most standard experiments with animals, our experiments have relied on the process of operant conditioning mediated by positive reinforcement. However, to achieve a proper effect, behaviour and reward must be adequately paired, which was of course also the case in our experiments. A very common tool, as well for pet rodents as for scientific purposes, are the activity wheels, respectively the tread mills which are voluntarily used to an extensive rate by many rodents and therefore seem to provide some rewarding entertainment to the animals. Unfortunately, we did not consider that in some way running on the ball is in itself a reward. This led to the effect that we had a reward which was paired with the desired behaviour and another which was not. When the animal was confronted with a task which was too complex or when too many novelties were introduced at one time, the paired reward was neglected and the animal obtained its reward simply by running. As an example, I would like to refer to Experiment 10, in which we were not able to train the animals to perform the landmark navigation task, but ended up with all animals running towards a fixed direction instead. This behaviour was, from my point of view, not only driven by a preference for a certain direction. This preference must also have been evoked by a certain motivation, which was the rewarding running. That these two effects can interact and therefore influence the training procedure is not hard to guess. My only suggestion how this second reward can be hindered from gaining too much influence is to keep the training easy enough to sufficiently provide the animals with the appropriate reward.

4.3 The contrast influences

Another series of experiments have dealt with the animals' ability to discriminate contrast. Those attempts revealed their general ability to perform such discriminations, but all of these experiments were dominated by instant preferences of the animals which might have been due to the contrast of the objects in the experimental environment. The results of Experiment 11 (Chapter 3.9), together with the results of Jan Regler's diploma thesis, give an idea of how delicate these preferences can influence an experiment. The results from the first discrimination task in Experiment 2 (Chapter 3.3) do not count in this respect, for the reason of its failure was not the different contrast of the targets; it was, in my opinion, the squared arrangement of the columns. In Experiment 11, on the other hand, the preference for the white columns appeared instantly. Although I was able to train the animals also to run towards the dark columns, the time effort of 50 days indicates that we deal with a very resistant effect. In

Experiment 14 (Chapter 3.13), where naive animals were trained under the same conditions as in Experiment 11, the same preference was observable. Unlike in Experiment 11, this effect could not be explained by experiences which the animals had made in recent experiments. It must therefore have been elicited by the properties of the environment itself. The continuative experiment of Jan Regler shed a new light on certain properties which we suspected to be responsible for the observed effects. He revealed that the brightest element in the environment also tends to be the most attractive. However, he also found that the attractiveness of an object also depends on its contrast against the background. One group of his animals was attracted to black coloured columns which were located in a bright environment, which supports this theory. The other group, on the contrary, did not show this tendency, which means that there must be even more factors which cause these preferences. Regrettably, we were unable to give a full account of the origin of these preferences. Albeit this problems, Mr Regler was successful in compiling a data set which gave us guidelines on how strong contrasts have to be to ensure a proper differentiation. There are other psychophysical features of the setup which are also of big interest, such as for example the minimal spatial acuity which the animals can discriminate in the setup. Nevertheless, before more attempts will be made in this matter, it would be desirable to dispose of a discrimination task which is more reliable and effective than the one which provided this data. An environment comparable to the ones used in Experiments 12-b (Chapter 3.11) and 18 (Chapter 3.17) would probably be more successful if, instead of columns, the walls were used to present the two patterns which have to be discriminated.

4.4 Immersiveness

If the animals perceive and interpret the environments which are presented to them as spatial was a question which many of our experiments tried to answer. Although the findings of Experiments 12-b and 18, paired with the finding of place cells which correlate with the virtual environment in Experiment 15 (Chapter 3.14), support our theory that the animals are able to perform spatial tasks in a virtual environment, we were not able to give evidence on how much the animals are immersed in the virtual environment. A measure of how real the animals perceive the simulation presented to them, could only be given by tasks which test if they can be deceived by the simulation. Such a test could for example examine if they retreat from a virtual insurmountable gap in the ground or if they flee from a virtual cat. Although we did not perform such specific tasks, we could observe very early that the animals do not flinch from running trough walls, which led us to the introduction of the column paradigm. This is of course due to the lack of a force feed back, without which the animals encountered only penetrable objects and therefore probably got used to neglecting an initial shyness from running trough walls. At the beginning of Experiment 18, we could observe that in case an animal came close to a wall, it reduced its speed, but did not refuse to run trough the walls all together. On the one hand, this suggests that the animals probably perceive the wall as an obstacle but, on the other hand, it also supports the theory that they quickly suppress this impression or that it is not strong enough to influence their behaviour further. To fully clear this question, a series of experiments are imaginable; however, I would not recommend implementing further investigations of spatial behaviour on our setup. As mentioned in Experiment 18, I think that with his experiment, the maximal complexity is reached which can be realized in our setup.

4.5 Task complexity

The real world equivalent of the tasks which were performed in our V.R. setup are all rather simple tasks which can be learned by a normal, healthy rat within a few days. In contrast to this, we were often compelled to apply a multiple of the training time which is reported for real world tasks. The first point that lies at hand is the fact that the animals have to deal with a fairly complex setup and although they get used to some of its elements during the handling procedure, some of the performance gets lost because the animals need some days to get familiarized with the whole procedure.
Another – and in my eyes the strongest – cause for the extended learning phases arises from the cue conflicts which appear in the setup. The only senses which are addressed by our setup are vision and proprioception, all the other deliver uncorrelated or in the worst case conflicting cues. Smells, sounds and even a smaller part of the visual field do not move according to the movements of the animal. The vestibular sense changes from correlated when the animals moves to uncorrelated when it starts or stops since the acceleration and deceleration information is missing. The vibrissae senses are most likely correlated since they detect the movements on the sphere and have no other contacts. From this variation of senses, the animals must now select those pieces of information which are correlated to the simulation and can provide them with cues to reach a reward. This process complicates the learning of a task considerably. Creating a setup which addresses all senses in an appropriate way seems to me as impossible as it is for human V.R. systems.
Although the animals run quite normally when mounted on the sphere, there is also a certain component of motor skills which they have to learn. Especially when they are forced to perform directed movements towards a goal, the animals have to improve their accuracy of interaction with the sphere. This learning process can be seen in the results of Experiment 9 (Chapter 3.7), where the performance improvement did not only result from the fact that the animals learned the task, but also from the fact that they learned to interact with the sphere, which can be seen from the increasing distances they were able to cover. If the performance on the sphere would have been as easy as the running in an activity wheel, the distances should have been the same over the days.
The design of the setup was intended to keep the action perception loop as close as possible; in fact, some of its elements are more accurate than in comparable V.R. devices for humans. The projection system, together with the graphic engine, provided image refreshment rates of up to 80 Hz, while the movements of the sphere were captured with a rate of 1500 Hz. But even under these precautions, errors can occur, for example when the animal slips slightly on the sphere. This can lead to inconsistencies which impair the action perception loop and, although if they are not perceptible, have an effect on the animals performance.
An intensely discussed problem in the research with virtual reality is simulator sickness, which we neglected completely in our setup. Due to the small number of experiences with animals in V.R., it is not known if this sickness can also occur in rats. As mentioned in Chapter 1.6, simulator sickness is related to motion sickness, which can be elicited in many animals and also in rats. The various factors which promote simulator sickness (described in Kolasinsky (1995) are also present in our setup; the above mentioned inconsistencies are one of them. Since we never controlled if some of the animals suffered from nausea in our setup, we can not prove its effect, but if it did indeed occur, this could be an explanation for the huge variations in the animals' performances and also for the prolonged learning. Some inconsistencies are also given by the visual system of the animals. The visual acuity of the rat is about 1 cycle per degree (see Chapter 1.3.2), which means that the projected image which is presented to the rat on the screen in a distance of 80 cm is blurred, compared to our vision. Under these circumstances small movements can be concealed in the blur of the vision. Due to this effect, the action perception circle is also interrupted as the animals have to move a

certain distance until they are able to perceive the motion on the screen. We tried to moderate it by using very big objects; however, the size of an object has no great effect on the motion parallax or the amount it looms during movements.
It remains unclear which of these effects actually took place or which made the interaction with the setup such a complex task and therefore prolonged the learning phases. Nonetheless, even if we knew them exactly, little could be done to prevent them since they are unavoidable inconveniencies which appear regularly in virtual reality.

4.6 Future experiments

As mentioned above, I did not find it fruitful to continue with the use of the setup for the investigation of long range spatial navigation behaviour. The efforts it costs and the problems which had to be solved to complete the water-maze task leave little hope that it is possible to advance towards more complex tasks. Nonetheless, the setup proved to be suitable for electrophysiological experiments and can therefore still help to answer questions about mechanisms of spatial behaviour. In most of the electrophysiological experiments, the animals' task simply is to explore the environment. This can easily be realised in our setup, the only requirement is probably an improvement of the punishing device to keep the animals within a certain arena. Combined with these methods, using V.R. still has the advantages that environments can easily be shifted, changed with respect to various parameters or simulate ranges beyond the dimensions of a laboratory. Although wireless mountable transducers for electrophysiological signals are meanwhile available which allow measurements over longer distances, the other benefits of the setup still remain. It is clear, though, that more data of cells need to be collected first, which allow us to make comparisons between the characteristics of place cells in virtual and in real world experiments.
Another experiment which should be enhanced is the discrimination task. Although we encountered many problems in realising these tasks, I am still confident that they can be performed successfully with our setup. With such a tool at hand, many psychophysical attempts could be realised. When combined with lesions or chemical treatment, these experiments can also be extended towards investigating the role of several molecular factors and brain regions which influence and mediate learning processes. Another method which can be included in these studies is micro dialysis, which also requires the connection of the animals with an analysis device and can therefore benefit from the fact that in our setup animals can move without being dislocated.
Finally, I will make some suggestions on technical details which could be improved for further experiments. The material of the screen, which at the moment consists of white cotton, could be replaced by a material with better reflective characteristics, as it is used for cinematic purposes. If the screen was made of two halves opening sideways like a cupboard, the necessity to raise the screen would be obsolete, which would probably allow us to narrow the gaps on the top and at the bottom of the screen. The three wires which keep the animals on top of the sphere could be replaced by less prominent thin wires receiving their necessary rigidity from tension. This would also require fixating the complete setup in a solid frame to which this tension could be applied. By these changes, the quality of the projection could be improved and the amount of visual field which is covered by the projection could be enlarged. This would also mean that the amount of elements which do not correspond to the projection could be reduced.

This leaves many opportunities for further work and I am positive that the problems which troubled our first attempts when introducing the V.R. technique to rat behavioural studies can be overcome.

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