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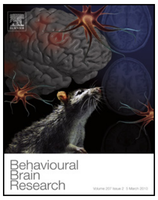

Research report

Mongolian gerbils learn to navigate in complex virtual spaces

Kay Thurley[a,b,*], Josephine Henke[a,1], Joachim Hermann[a,b,1], Benedikt Ludwig[a,1], Christian Tatarau[a,1], Aline Wätzig[a,1], Andreas V.M. Herz[a,b], Benedikt Grothe[a,b], Christian Leibold[a,b]

[a] Department Biologie II, Ludwig-Maximilians-Universität München, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany
[b] Bernstein Center for Computational Neuroscience Munich, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany

HIGHLIGHTS

- We implemented complex virtual realities to investigate rodent navigation.
- Mongolian gerbils were successfully trained to navigate in such virtual environments.
- The animals generalized to unknown environments after training on a different maze.

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ABSTRACT

Virtual reality (VR) environments are increasingly used to study spatial navigation in rodents. So far behavioral paradigms in virtual realities have been limited to linear tracks or open fields. However, little is known whether rodents can learn to navigate in more complex virtual spaces. We used a VR setup with a spherical treadmill but no head-fixation, which permits animals not only to move in a virtual environment but also to freely rotate around their vertical body axis. We trained Mongolian gerbils to perform spatial tasks in virtual mazes of different complexity. Initially the animals learned to run back and forth between the two ends of a virtual linear track for food reward. Performance, measured as path length and running time between the virtual reward locations, improved to asymptotic performance within about five training sessions. When more complex mazes were presented after this training epoch, the animals generalized and explored the new environments already at their first exposure. In a final experiment, the animals also learned to perform a two-alternative forced choice task in a virtual Y-maze. Our data thus shows that gerbils can be trained to solve spatial tasks in virtual mazes and that this behavior can be used as a readout for psychophysical measurements.

1. Introduction

Rodents are the most widely used model animals for studying spatial learning and navigation [1–3] and the underlying neuronal processes [4–9]. Traditionally, spatial behavior has been investigated in these studies using linear tracks [10] or open fields in various enclosures [11]. Building mazes for more complex spatial tasks [8,12] is possible with much greater effort but does not overcome the restrictions of the typical lab scale of a few meters. More recently, virtual reality (VR) paradigms have been developed [13–18], which not only make it feasible to investigate behaviors on arbitrary spatial scales but are also suitable for closed-loop manipulations of the environments, and even allow one to generate physically impossible environments to discriminate between alternative navigation strategies [for reviews see 19,20]. Such VR setups allow one to use stable head-fixed preparations in awake behaving animals and combine navigational experiments with advanced recording techniques, such as intracellular recordings [14,15] or two-photon imaging [21,22]. In spite of the great success of these VR setups, there were only few attempts to train animals to carry out more complex navigational tasks in virtual environments [23,24]. Behavioral paradigms in virtual realities for rodents mostly made use of open fields [13], linear tracks [14,15,17,25] or were limited to providing optical flow [16,22] and spatially unrelated visual stimuli [26]. Moreover, subtle differences in the neuronal space codes have been reported between running in real worlds

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[*] Corresponding author at: Department Biologie II, Ludwig-Maximilians-Universität München, Germany. Tel.: +49 89218074823; fax: +49 89218074803.
E-mail address: thurley@bio.lmu.de (K. Thurley).

[1] Contributed equally, in alphabetical order.

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and VR behavior [18] such that it is not entirely resolved, whether the behavior observed in VR is based on the spatial strategies to a similar extent than in real worlds or whether it is more strongly reflecting direct sensory (visual) stimulation.

In this paper, we report on virtual spatial behaviors in more complex environments, in which the animals are required to perform navigational or behavioral tasks. Since VR setups mainly stimulate the visual modality, we used Mongolian gerbils (*Meriones unguiculatus*) whose visual system is superior to those of mice or rats [27–29]. Moreover, spatial navigation in gerbils has been well documented in studies on path integration [1,30,31]. Our results demonstrate that gerbils which learned to operate a virtual linear maze were able to make use of their acquired skills in more complex virtual environments. There, the animals exhibited exploratory behavior, even upon first exposure.

2. Methods

2.1. Animals

Experiments were performed on adult Mongolian gerbils (*Meriones unguiculatus*). We used a total of ten gerbils of both sexes. Training started at ages between three and seven months and the animals weighed between 70 and 100 g. The animals received a diet which kept their weight at about 85–90% of their free feeding weight. All experiments were approved according to German Animal Welfare Act and linked European regulations (Reg. von Oberbayern, AZ 55.2-1-54-2532-10-11).

2.2. Experimental apparatus

As in previous approaches [13,14], the animal is held on the north pole of a Styrofoam sphere which itself sits in an aluminum bowl and floats on an air stream that is directed into the bowl from below (Fig. 1A and B). The sphere's weight is adjusted to 150 g to match the torque of an animal with 100 g body weight. The force $(I_\bullet\,\dot{\omega})/R_\bullet$ the animal exerts on the sphere (radius: $R_\bullet$, moment of inertia: $I_\bullet$) during an angular acceleration $\dot{\omega}$ is thereby supposed to equal the force $m_a\,\dot{v}$ the animal (mass: m_a) would provide to the ground during acceleration $\dot{v}$, i.e.,

$$m_a\,\dot{v} = \frac{I_\bullet\,\dot{\omega}}{R_\bullet} = \frac{I_\bullet}{R_\bullet^2}\,\dot{v}.$$

This equality constrains the sphere's moment of inertia by the animal mass. Given the moment of inertia of a hollow sphere $I_\bullet = (2\,m_\bullet\,R_\bullet^2)/3$, we can hence derive the mass $m_\bullet$ of the hollow sphere as

$$m_\bullet = \frac{3}{2}\,m_a.$$

The animal is positioned on the sphere with the help of a custom-made harness that leaves head and legs freely movable. The fixation is designed such that the animal can freely rotate around its vertical body axis. To attach the animal to the handle above the sphere we make use of a magnetic mechanism, which permits flexibly placing the animal in the setup and taking it out again (Fig. 1A, bottom-left corner). Forward and backward movements of the animal induce rotations of the sphere similar to a treadmill. Rotations of the sphere in the azimuthal plane are hampered by two little wheels mounted at the rim of the aluminum bowl that contains the sphere. The sphere's movements are detected by two infrared sensors (conventional optical USB computer mice) and fed into the computer that generates the visual VR. The VR is projected by a video projector (Sanyo PLC-ET30L with custom-mounted Sanyo LNS-T11 objective) via a mirror system consisting of a planar and a rotational symmetric aspherical mirror onto a 360° toroidal projection screen, generating the full circle image on the toroidal screen. The mirror was milled and polished (Kugler GmbH, Salem, Germany) according to an optical model of the geometry of the setup [32]. Animals are rewarded with a high energy paste (Nutri-plus gel, Virbac, Bad Oldesloe, Germany) that is administered via a small flexible plastic tube connected to an automatic feeder (see Supplementary material for details). The reward apparatus is controlled by the VR software.

2.3. Simulation of the virtual environments

We generated our mazes with the open-source software Blender (v2.49, http://www.blender.org/). Blender can be accessed via a Python API, which allows for automated maze generation. From Blender we exported the 3D data to a VRML (Virtual Reality Modeling Language) file – a format for representing 3D vector graphics. For real-time rendering and simulation of the visual properties of the environments based on these VRML files, we used Vizard Virtual Reality Toolkit (v3.18, WorldViz, http://www.worldviz.com). Synchronization between Vizard and the experimental apparatus was managed by writing the infrared mouse data into shared computer memory using custom-made software based on the libusb-win32 library (v1.2.4, http://sourceforge.net/apps/trac/libusb-win32/wiki). Virtual collision detection was accomplished by the VR software and resetting the infrared mouse data accordingly. When an animal tried to run through a virtual wall, the spatial updating of the projection stopped but the treadmill was not blocked and the animal could still move. The sphere's rotation was not affected by a virtual collision. In initial sessions animals generally kept on running "into the virtual wall" regardless of the halted projection and eventually stopped running, turned by some angle and again started running. After training, they reoriented themselves after a virtual collision to be able to run parallel to the virtual walls and perform the navigational task.

We used four different types of mazes: a linear maze, a U-shaped maze, a maze in which the animals had to run circular trajectories, and a Y-shaped (decision) maze (Fig. 1C). The design of the maze walls was based on Harvey et al. [14]. In addition to black, white and gray levels, we typically used green walls to visually identify areas where reward was delivered. Active reward areas were indicated with textures in bright green color. When the animal entered the area and was given its reward, the color switched to darker green and the next active reward site was switched to bright green. In the Y-shaped (decision) maze, rewards were delivered if the animal made a correct decision depending on what was presented at the end of the Y's arms (see Section 3 for details).

2.4. Behavioral training scheme

The training started with three to five days of handling and habituation to the experimenter, to the VR laboratory, and to wearing the harness. Afterwards the animals were familiarized for five to seven days to the VR setup, i.e., the fixation on the treadmill, the VR projection, and to the reward apparatus. The familiarization was done in the linear maze and with a reduced projection screen of 270°, to allow the experimenter to manually access the animal. Each session lasted about fifteen minutes. When the animals started moving on the treadmill and paid attention to the projection, we closed the projection screen (360°) and the actual training on the linear track began. All animals underwent this block of linear maze training. When performance approached its optimum (see Section 3 for details), the animals were introduced into different follow-up experiments: (1) rotation invariance, (2) U-shaped maze, (3) circular maze, (4) decision task. Some animals were tested