Published in final edited form as:

J Neurosci Methods. 2009 June 30; 181(1): 82–88. doi:10.1016/j.jneumeth.2009.04.023.

The Floor Projection Maze: A novel behavioral apparatus for presenting visual stimuli to rats

Sharon C. Furtak^a, Christine E. Cho^b, Kristin M. Kerr^b, Jennifer L. Barredo^b, Janelle E. Alleyne^{a,c}, Yolanda R. Patterson^{a,c}, and Rebecca D. Burwell^{a,b,*}

^a Department of Psychology, Brown University, Providence, RI 02912, USA

^b Department of Neuroscience, Brown University, Providence, RI 02912, USA

^c Tougaloo College, Tougaloo, MS 39174, USA

Abstract

There is a long tradition of studying visual learning in rats by presenting stimuli vertically on cards or monitors. The procedures are often labor intensive and the rate of acquisition can be prohibitively low. Available evidence suggests that rats process visual information presented in the lower visual hemifield more effectively than information presented in the upper visual hemifield. We capitalized on these findings by developing a novel apparatus, the Floor Projection Maze, for presenting visual information directly to the floor of an exploratory maze. Two-dimensional (2D) visual stimuli were presented on the floor by back-projecting an image from a standard digital projector to the semi-transparent underside of the floor of an open maze. Long-Evans rats rapidly acquired easy 2D visual discriminations (Experiment 1). Rats were also able to learn a more difficult shape discrimination in dramatically fewer trials than previously reported for the same discrimination when presented vertically (Experiment 2). The two choice discrimination task was adapted to determine contrast sensitivity thresholds in a naïve group of rats (Experiment 3). Contrast sensitivity thresholds were uniform across three subjects, demonstrating that the Floor Projection Maze can be used for visual psychophysics in rats. Our findings demonstrate that rats can rapidly acquire visual tasks when stimuli are presented horizontally on the floor, suggesting that this novel behavioral apparatus will provide a powerful behavioral paradigm in the future.

Keywords

Vision; Two-dimensional stimulus; Automated; Rat; Psychophysics

1. Introduction

The use of rodent models to study human cognition has been hampered by cross-species differences in sensory processing needs. In particular, humans tend to rely more than rodents do on visual information and less on olfactory and tactile information. Yet, there is a long history of research on the visual capabilities of rats. Although visual acuity in rats is much lower than in humans, there is considerable evidence that visual stimuli can control the behavior of rats. Indeed, in the 1930s and 1940s, Lashley produced a landmark series of 15 research articles on the mechanisms of vision in rats beginning with the introduction of his Jumping Task (Lashley, 1930a).

*Corresponding author at: Brown University, 89 Waterman Street, Providence, RI 02912, USA. Tel.: +1 401 863 9209; fax: +1 401 863 1300. rebecca.burwell@brown.edu (R.D. Burwell).

The rat eye is a typical mammalian eye, and in most respects, the rat visual system is a typical mammalian visual system, including primary and secondary visual cortex as well as all the relevant subcortical structures. Studies show that rats have binocular vision (Lashley, 1934), detect motion (Douglas et al., 2006), and show robust contrast sensitivity (Keller et al., 2000). Although the acuity of rats is lower than that of humans or monkeys, they are able to see well enough to distinguish behaviorally relevant stimuli. Pigmented rats demonstrate acuity of about 1.0 cycle per degree (cpd Prusky et al., 2000, 2002; also see Lashley, 1930a; Birch and Jacobs, 1979). In practical terms, pigmented rats are able to discriminate approximately eight lines per centimeter at a distance of 7 cm. Unlike the human eye, the rat eye lens is not flexible and does not accommodate. The unaccommodated rat eye has a depth of focus of 7 cm to infinity (Powers and Green, 1978), whereas the unaccommodated human eye has a depth of focus of 3 m to infinity.

The first successful method of training rats to discriminate between two-dimensional (2D) visual stimuli was Lashley's Jumping Task (Lashley, 1930b). In this task animals jumped toward stimuli, falling to a net if they jumped toward the incorrect stimulus and safely reaching a platform if they jumped toward the correct stimulus. Although the method demonstrated that rats can solve discrimination problems, rate of acquisition is low, and there are obvious drawbacks for modern research. Since the mid-1990s several groups have developed more modern behavioral procedures for training and testing rodents on tasks that use 2D visual stimuli. These approaches rely on standard environments for testing rats, including dry mazes (Gaffan and Eacott, 1995; Forwood et al., 2007), elongated water mazes (Prusky et al., 2000, 2002), and operant chambers (Bussey et al., 1997). Each approach has advantages and disadvantages for testing stimuli in rats. Notably, in all of these tasks, the visual stimuli are presented vertically on the walls of the apparatus.

There is considerable evidence that rats may be more prepared to process visual information presented horizontally on the ground. Lashley (1938) showed that when rats were trained on a patterned stimulus and then tested with only part of the stimulus exposed, rats were at 95–100% accuracy when the lower half, lower left quadrant, or lower right quadrant of the stimulus was exposed. In contrast, accuracy was at chance, 40–50%, when the upper half, upper left quadrant, or upper right quadrant was exposed. Lashley (1932) also provided evidence that ganglion cells are more concentrated in the upper hemifield of the retina in laboratory rats, suggesting that acuity is better in the lower half of the visual field. This distribution of photoreceptors is consistent with observations that rats tend to rely on local luminance differences in the lower half of the visual field (Minini and Jeffery, 2006). Other research suggests that hippocampal place fields in the rat are modulated more by the appearance of exploratory maze floors than by the appearance of the walls (Jeffery and Anderson, 2003).

Taken together, available research suggests that rats might process visual information more effectively if presented on the floor. For the present series of experiments, we tested this hypothesis by presenting stimuli on the horizontal surface, or floor, of an exploratory maze. We designed a novel apparatus, the Floor Projection Maze, that displays 2D stimuli to the floor of an open maze via back-projection from a standard projector to an angled mirror located below the maze floor.

In three experiments we were able to demonstrate that pigmented Long-Evans rats can rapidly solve 2D visual discriminations when the stimuli are projected to the floor. We also demonstrated that this approach can be used for visual psychophysics in rats.

2. Material and methods

2.1. Subjects

Subjects were six male Long-Evans rats (Charles Rivers Laboratories, Wilmington, MA). Three animals were used as subjects in both Experiments 1 and 2. Three naïve animals were used in Experiment 3. Animals were placed on a feeding schedule prior to behavioral training to maintain body weight at 85–90% of free feeding weight. Animals were handled daily for 1 week prior to behavioral training. Subjects were single housed in a 12:12 light–dark cycle with *ad libitum* access to water. All procedures were in accordance with the appropriate institutional animal care committee and NIH guidelines for the care and use of animals in research.

2.2. Apparatus

The behavioral chamber, the Floor Projection Maze, was an 81.3 cm square open field maze with a clear Plexiglas floor (1.2 cm thick) and modular white Plexiglas walls (33 cm height, 0.6 cm thick; Fig. 1). The underside of the floor was lined with Dual Vision Fabric (Da-Lite Screen Company, Warsaw, IN), a unity gain flexible fabric designed for rear projection with standard LCD and DLP projectors (Fig. 1). To back-project to the maze floor, we used an LCD projector (1200MP projector, Dell, Inc.) directed to a mirror located under the maze floor at 45° relative to the floor (Fig. 1). The structure holding the maze and mirror was constructed of wire shelving (Nexel Wire Shelving, Superior Shelving Co., Tacoma, WA) and aluminum framing (80/20, Inc., Columbia City, IN).

The maze was interfaced with three computers, one for tracking, one for data acquisition, and one for behavioral control. Tracking was accomplished with a CinePlex Digital Video Recording and Tracking System (Plexon, Inc., Dallas, TX) that uses an Imaging Source™ camera (640 × 480 resolution, 30 frames per second) interfaced with a computer running Windows XP. The digital video camera was positioned 88.9 cm above the maze floor and provided a live-image aerial view of the chamber. Animals were tracked in the maze by contrast. A centroid was calculated such that a single point in the center of the animal's back yielded *x* and *y* coordinates. Time-stamped *x* and *y* coordinates of animal position and occurrence of behavioral events were provided in real time to a Multichannel Acquisition Processor (MAP, Plexon, Inc.) and collected by SortClient (Plexon, Inc.). Custom Matlab programs (Mathworks Inc., Natick, MA) translated the *x* and *y* coordinates into behaviorally relevant positions and relayed them to a computer dedicated to behavioral control using Med-PC IV software and Med Associates hardware. The third computer controlled all behavioral equipment with a DIG-716P2 Smart Control Output Interface (Med Associates, Inc.), including reward delivery, auditory stimuli, and floor images with custom-written software in Med PC. The behavioral computer was interfaced with the MAP computer by commercially available equipment including a DIG-700P2 PCI Interface Connection Card, a DIG-716P2 SmartCtrl 8 Input, 16 Output card, and a DIG-726TTLx SuperPort 16 Output Module (Med Associates, Inc.).

Food reward was delivered by pellet dispensers or automated pumps (Med Associates, Inc., St. Albans, VT), to custom food ports located in the wall at the end of the maze (Fig. 2A). Food reward was 40 mg food pellets (Bioserve, Frenchtown, NJ) for Experiments 1 and 3, and chocolate flavored non-fat milk solution for Experiment 2. All auditory stimuli were delivered through a speaker controlled by an auditory stimulus generator (ANL926, Med Associates, Inc.), which was located on the wire shelf above the maze. For Experiments 2 and 3, an 18 cm long clear plastic divider (33 cm in height) was positioned in the center of the North end of the maze during both training and testing procedures (see Supplemental Material).

For Experiment 1, tracking was unreliable, thus an operator observed subjects on a monitor and signaled the Med Associates software by pressing one of three buttons. Button one was pressed when a subject entered the ready position box (see below), button two was pressed when the subject entered the location of the left stimulus, and button three was pressed when the subject entered the location of the right stimulus. All other aspects of the task were automated. For Experiments 2 and 3, a software update for CinePlex rendered tracking completely reliable, and the task was fully automated.

2.3. Experiment 1: easy 2D visual object discrimination

Rats were trained on a visual discrimination task in which pairs of 2D stimuli were presented on the floor of the exploratory maze. In all phases of shaping and training in which pairs of stimuli are presented, presentation of the correct stimulus was counterbalanced for left and right. Testing was preceded by a number of shaping steps such that rats were trained to approach the correct stimulus for a food reward. Subjects were 2 months of age at the time of testing. For all experiments, special care was taken to design stimuli that were well within the limits of visual acuity for Long-Evans rats (Jacobs et al., 2001; Douglas et al., 2005).

2.3.1. Pre-training—Prior to training, animals were habituated to the apparatus and introduced to food pellet rewards. A grey floor was maintained throughout the session. At the start of a trial, white noise was presented for a variable period (mean, 20 s; range, 10–30). Offset of the noise was followed in 500 ms by delivery of a food pellet to the food port in the center of the South wall. Food delivery was followed by a variable intertrial interval (ITI, mean, 20 s; range, 10–30).

2.3.2. Shaping—In the *first* phase of training (*Shape 1*), animals learned to approach a 2D visual object (a black square with a white plus in the center) presented on the floor for a food reward. Each trial began with the onset 70 dB white noise and the presentation of a 2D object in the North of the maze on a grey background. When an animal approached the object, the white noise was terminated and food was delivered. If the object was not approached in 1 min, the trial was terminated, and food was presented on a 50% probability. The ITI was pseudo-randomly varied between 10, 20, and 30 s with a mean of 20 s.

In *Shape 2*, animals learned to approach a location in the center of the open maze prior to presentation of a 2D visual object, a black square with a white plus in the center. This location is termed the “ready position”. Animals were trained to approach the ready position from the direction of the food port such that they were facing the location at which the stimuli would appear. They were required to remain still in the ready position until stimulus presentation. If animals approached the ready position from any other direction, stimuli were not presented. This procedure allowed for control over the direction in which the rat approached the stimuli as well as the distance at which the animal would view the stimulus presentation. A grey floor was presented, white noise was initiated, and the ready position stimulus was presented (black square, Fig. 2A). When the animal advanced to the ready position, the white noise terminated, and the training object was presented. When the animal approached the object, the object disappeared, and the food reward was delivered. At the end of the variable ITI, white noise indicated the start of the new trial. If the ready position or the object was not approached in 1 min, the trial terminated.

In *Shape 3*, animals learned to discriminate between two objects. A grey floor was presented during the ITI. At the end of the ITI, white noise and the ready position stimulus were presented. When the animal advanced to the ready position, the white noise was terminated, and a pair of objects was presented, the familiar stimulus (a black square with a white plus in the center) and a new stimulus (a black square of the same size with a white circle in the center; Fig. 2A,

right). The familiar, plus, stimulus was the rewarded one. Approach to the correct stimulus was followed by food reward. The trial terminated if the animal approached the incorrect stimulus. At the end of the variable ITI, white noise indicated the start of the new trial. If the ready position was not approached in 1 min, the trial terminated. Once an animal reached criterion of 10 consecutive correct choices, it was advanced to the testing phase.

2.3.3. Testing—Animals were tested on pairs of objects designed to resemble dice (Fig. 2B). Otherwise, the task was the same as the final phase of shaping. Three dice problems were presented successively: 1 vs. 4; 5 vs. 2; and 6 vs. 3, such that the first number of each pair was the correct choice. After the third dice discrimination problem, rats were trained on a blue–yellow color discrimination using the same parameters (Fig. 2C). For both the dice stimuli and the color discrimination, learning was operationally defined as 10 consecutive correct trials.

2.4. Experiment 2: difficult 2D visual object discrimination

Following completion of the dice task, the same animals ($n = 3$) were used to pilot a number of experimental designs over several months. At about 14 months of age, animals were reshaped on the circle-plus discrimination with the plus as the rewarded stimulus. They were then tested on a new and more challenging pair of stimuli that consisted of a triangle and a square (Fig. 2D). The triangle and square were equiluminant and equal in area. The triangle was correct (rewarded) and the square was incorrect. The task parameters were the same as for Experiment 1 with the following exceptions. Rats were required to remain at the ready position for 500 ms before stimulus pairs were presented, a correct choice was signaled by a tone–food reward, and the variable ITI was a mean of 10 s and ranged from 5 to 15 s. Use of the tone to signal food allows better control over behavior when the response to be rewarded is distant from the food port. As before criterion was 10 consecutive correct trials. For this experiment, tracking was fully automated. Food reward was a chocolate milk solution rather than a food pellet. This alteration was made because liquid dispensers can be located anywhere in the maze with minor alterations. The liquid reward also avoided chewing artifact that was evident in parallel single unit recording studies.

2.5. Experiment 3: contrast sensitivity threshold task

In Experiment 3, we evaluated the Floor Projection Maze for testing contrast sensitivity in rats. Apparatuses currently in use have certain limitations. One approach employs a water task (Prusky et al., 2000). Acquisition is fairly rapid, but the task cannot be automated and is not amenable to physiological recordings. Another approach automated presentation of vertical stimuli, but acquisition is slow (Keller et al., 2000). The task described here overcomes the disadvantages of the two other behavioral paradigms currently being used for contrast threshold detection in rats by using a dry chamber in which two stimuli are projected onto the floor. Subjects were three naïve male Long–Evans rats that were 3 months of age at the time of testing. All shaping and testing procedures were fully automated.

2.5.1. Pre-training—Prior to training, animals were habituated to the apparatus and trained to associate delivery of a food pellet to the food port with a 70 dB, 15 kHz. Rats were considered to have learned the association when they reliably approached the food port in response to a tone.

Animal then underwent a series of shaping procedures. For all phases of shaping, rats were advanced to the next phase after reaching a criterion of 10 consecutive correct choices, not including omitted trials. In the *first* step of training (*Shape 1*), animals learned to associate a particular 2D stimulus (presented on the floor) with a tone–food reward. Two stimuli were projected on the floor at the north end of the chamber, a black square with a white dot in the center and a black square of the same size without a dot. The rat received a tone–food reward

when it approached the rewarded stimulus (the dot stimulus), and the trial terminated when the rat approached the unrewarded stimulus. If the animal did not approach either stimulus within 30 s of presentation, the trial was terminated and considered to be an omitted trial.

In *Shape 2*, animals were required to approach the ready position stimulus (Fig. 2A, center) from the south and remain at that location for a variable duration in order to receive a reward. The duration was gradually increased from 300 to 600 ms. Tone-food reward was delivered if the rat approached the ready position from the south and remained there for the expected duration. Trials were terminated if the rat approached the ready position from a direction other than the south or left the ready position early. Trials were defined as omitted if the rat did not approach and remain at the ready position, or did not make a choice in 30 s.

In *Shape 3*, approach to the food port initiated the start of a trial at which point the ready position stimulus appeared. Animals were required to approach the ready position from the south and remain for 600 ms, upon which a pair of 2D objects appeared and the intensity of white noise was reduced to 65 dB. Approach to either stimulus signaled the subject's choice. A correct choice was followed by a tone-food reward. An incorrect choice resulted in trial termination. If the rat did not approach the ready position within 30 s, did not remain for 600 ms, or failed to make a choice in 30 s, the trial was considered to be omitted.

Shape 4 was the same as *Shape 3*, except that the stimulus pairs were replaced by circular stimuli (12.5 cm, projected diameter). One stimulus was a homogeneous grey circle, and the other was a circle with sinusoidal gratings at 0.13 cycles, with the grating circle set as the correct stimulus. At the start of the task, the contrast of the sinusoidal gratings was 100% (Fig. 2E). The orientation of the gratings was such that the orientation of the grating was 45° in reference to vertical for the rat's line of sight. In this and all subsequent phases of shaping and testing, the grating was presented equally often on left and right.

2.5.2. Testing—Pilot work indicated that the rats were more erratic on the first 20–30 trials of a session. Thus, prior to testing rats were trained with discriminations on which the correct stimulus was at 100, 50, or 25% contrast varied pseudo-randomly (Fig. 2E). Testing began when the rat was performing reliably.

Contrast sensitivity thresholds were obtained using a stair step procedure such that four correct choices led to a step down in contrast and two incorrect choices led to a step up in contrast. There were 13 contrast levels in the testing paradigm: 50, 25, 20, 18, 16, 14, 12, 10, 8, 6, 4, 2, and 1% (Fig. 2E). Steps were decreased to 2% increments beginning at 20% because pilot tests suggested that rats had a threshold between 18 and 10%. A step down followed by a step up was defined as a reversal. Threshold was the last level after five consecutive reversals.

2.6. Data analysis

Trial information was recorded by custom Med-PC IV software for subsequent analysis. Behavioral data is presented as mean $\pm$ S.E.M. Data are presented for individual subjects and no inferential statistics were performed.

3. Results

3.1. Experiment 1: easy 2D visual object discrimination

All three subjects completed shaping rapidly. Shaping was completed in seven ($n = 2$) or eight ($n = 1$) daily 30 min sessions.

Subjects quickly learned the first dice problem, and trials to criterion (TTC) decreased with each successive problem. On the first problem (1 vs. 4), animals reached criterion in a mean

of 83.00 ± 16.9 S.E.M. trials, including the 10 trials required to reach criterion (Fig. 3). On the second dice problem (5 vs. 2), animals reached criterion in 54.00 ± 9.8 S.E.M. trials. On the third problem (6 vs. 3) animals reached criterion in 16.67 ± 1.5 S.E.M. trials.

The same animals were then tested on a color discrimination problem (Fig. 2C). All subjects were performing at chance after more than 300 trials, and testing was terminated. It should be noted that for the color discrimination pair we chose the wavelengths were approximately 440 and 560 nm. Jacobs et al. (2001) showed that pigmented rats cannot discriminate colors of wavelengths when both are over 400 nm.

3.2. Experiment 2: difficult 2D visual object discrimination

After completion of the dice discrimination task, one of the rats was used to test tracking procedures in brief sessions for several tasks. Testing occurred on 7 days, but was never sufficient for the animal to acquire a task. A second rat performed 20 trials on a discrimination task over 2 days also to test tracking. The third rat was not tested during the intervening period. At 14 months of age, subjects were tested on a new discrimination problem, a white square vs. a white triangle of equal area. Subjects ($n = 3$) reached criterion on this more difficult discrimination problem in a mean of 521.33 ± 35.8 S.E.M. trials (Fig. 3, right).

3.3. Experiment 3: contrast sensitivity threshold task

Animals ($n = 3$) received one session per day. Subsequent to 30 tone-food pairings, animals received 1 session of *Shape 1*. This was followed by 4–6 sessions of on *Shape 2*. Animals then received shaping on new stimuli (the plus or circle stimulus on a square black background, but outlined in white). The addition of the white outlines was counter productive as subjects were not able to reach criterion in 9 days on the discrimination. The surrounding boxes were removed and each rat met criterion on *Shape 3* in 5–8 sessions.

Animals were then trained on *Shape 4*, the easiest level of the contrast discrimination in which a 100% contrast sinusoidal grating was the rewarded stimulus and a homogeneous grey circle was the unrewarded stimulus. Each rat required 2 or 3 sessions to reach criterion. At this point, each rat was tested for contrast sensitivity threshold by the stair step procedure, as described in Section 2. The contrast sensitivity threshold was identified as 12% for each of the three subjects.

4. Discussion

In this paper, we have introduced a novel behavioral apparatus, the Floor Projection Maze, which allows presentation of 2D visual stimuli directly to the floor of an open maze. Results from three visual tasks demonstrated that Long-Evans rats readily learn about 2D visual stimuli when presented on the floor. We showed that rats can rapidly solve two choice discrimination problems, and that the apparatus can be used effectively for visual psychophysics.

Interestingly, the rats learned the dice task more quickly than the triangle vs. square discrimination. There are several possibilities for the faster acquisition rate for the dice task. One difference is that the intertrial intervals were twice as long in the dice task as compared to the triangle-square task. With less interference from the prior trial, it may be that rats were able to learn more quickly. Second, in the dice task, rats were required to pass through the ready position, but not to maintain that location for any duration. Our experience is that training rats to remain still at the ready position slows acquisition, but not enough to account for the difference in acquisition rates between the dice task and the triangle-square task. A third possibility is that the dice task is inherently easier to learn because the stimuli can be discriminated on more dimensions, including number of objects, overall luminance, and

differences in all four quadrants of the stimuli to be discriminated. In contrast, the triangle and square differ only in shape. Despite the relatively greater number of trials required to learn the triangle-square problem as compared to the dice stimuli, our evidence suggests that acquisition was much faster when the triangle and square were presented on the floor than when presented vertically in prior studies (Minini and Jeffery, 2006). Consistent with Lashley (1938), Minini and Jeffery (2006) argued that rats tended to discriminate based on the lower portion of the vertical stimuli. Bussey et al. (2008) argued against this interpretation. However, all stimulus pairs shown in that study also differed in the lower part of the stimulus. With all possible explanations taken into account, our conclusion is that rats can acquire visual discriminations more rapidly when presented on the floor in an exploratory maze than when presented vertically either on the wall or an upright monitor.

We also demonstrated that the Floor Projection Maze can be used as an automated method for visual psychophysics in rats. Rats were trained on a grating at 0.13 cpd at 100% contrast. Each of three Long-Evans rats displayed peak sensitivity at 12% contrast. This finding is consistent with previous studies in the same strain in which stimuli were presented vertically. Keller et al. (2000) trained rats in an operant chamber procedure and found that at 0.12 cpd, peak sensitivity was at 15% contrast. One disadvantage of that approach is that the training is longer. In that study, learning to distinguish between the maximum contrast sinusoidal grating and the solid grey stimuli required 2 weeks of training, whereas in our study, rats required 2 or 3 sessions to distinguish between the grating and the grey stimulus. There are two disadvantages of our contrast sensitivity threshold procedure. One is that the two choice procedure requires that subjects look from side to side such that the orientation of the grating is varied. Second, the subjects are required to approach the grating to make a selection, which alters the spatial frequency. In future studies, we intend to solve both issues by using a go, no-go procedure such that the rat is required to approach the grey stimulus for a reward and refrain from approaching the grating. In that version, the grating will always be viewed from the same angle and distance.

5. Conclusion

The Floor Projection Maze affords a number of advantages over other approaches to visual testing in rats. Acquisition is rapid, procedures can be automated, and the approach can be modified for use in a multitude of applications and learning paradigms. Interchangeable walls allow for changes in the appearance or location of the walls and the food magazines. Inserts can be added to change the shape or size of the maze. The maze can easily be adapted to back-project to the walls. The Floor Projection Maze is amenable to single unit and local field potential recordings as well as additional technical methods. Notably, this is the first chamber of its kind to introduce the innovative feature of back-projecting images to the floor of an open maze. This feature takes advantage of the natural tendency of rats to attend to the floor of a chamber and may facilitate the performance of pigmented rats on visual tasks. These results confirm that this novel behavioral apparatus will provide a powerful behavioral paradigm in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was funded by NSF IOB-0522220 to RDB, NIH 1 T32 NS062443 to KMK, and NIH 1 T32 MH019118 to SCF.

References

- Birch D, Jacobs GH. Spatial contrast sensitivity in albino and pigmented rats. *Vision Res* 1979;19:933–7. [PubMed: 516464]
- Bussey TJ, Muir JL, Everitt BJ, Robbins TW. Triple dissociation of anterior cingulate, posterior cingulate, and medial frontal cortices on visual discrimination tasks using a touchscreen testing procedure for the rat. *Behav Neurosci* 1997;111:920–36. [PubMed: 9383514]
- Bussey TJ, Padain TL, Skillings EA, Winters BD, Morton AJ, Saksida LM. The touch-screen cognitive testing method for rodents: how to get the best out of your rat. *Learn Mem* 2008;15:516–23. [PubMed: 18612068]
- Douglas RM, Neve A, Quittenbaum JP, Alam NM, Prusky GT. Perception of visual motion coherence by rats and mice. *Vision Res* 2006;46:2842–7. [PubMed: 16647739]
- Douglas RM, Alam NM, Silver BD, McGill TJ, Tschetter WW, Prusky GT. Independent visual threshold measurements in the two eyes of freely moving rats and mice using a virtual-reality optokinetic system. *Vis Neurosci* 2005;22:677–84. [PubMed: 16332278]
- Forwood SE, Bartko SJ, Saksida LM, Bussey TJ. Rats spontaneously discriminate purely visual, two-dimensional stimuli in tests of recognition memory and perceptual oddity. *Behav Neurosci* 2007;121:1032–42. [PubMed: 17907834]
- Gaffan EA, Eacott MJ. A computer-controlled maze environment for testing visual memory in the rat. *J Neurosci Methods* 1995;60:23–37. [PubMed: 8544484]
- Jacobs GH, Fenwick JA, Williams GA. Cone-based vision of rats for ultraviolet and visible lights. *J Exp Biol* 2001;204:2439–46. [PubMed: 11511659]
- Jeffery KJ, Anderson MI. Dissociation of the geometric and contextual influences on place cells. *Hippocampus* 2003;13:868–72. [PubMed: 14620882]
- Keller J, Strasburger H, Cerutti DT, Sabel BA. Assessing spatial vision—automated measurement of the contrast-sensitivity function in the hooded rat. *J Neurosci Methods* 2000;97:103–10. [PubMed: 10788664]
- Lashley KS. The mechanism of vision. III. The comparative visual acuity of pigmented and albino rats. *J Gen Psychol* 1930a;37:481–4.
- Lashley KS. The mechanism of vision. I. A method for rapid analysis of the pattern-vision in the rat. *J Gen Psychol* 1930b;37:453–60.
- Lashley KS. The mechanism of vision. V. The structure and image-forming power of the rat's eye. *J Comp Psychol* 1932;13:173–200.
- Lashley KS. The mechanism of vision. VII. The projection of the retina upon the primary optic centers in the rat. *J Comp Neurol* 1934;59:341–73.
- Lashley KS. The mechanism of vision. XV. Preliminary studies of the rat's capacity for detail vision. *J Gen Psychol* 1938;18:123–93.
- Minini L, Jeffery KJ. Do rats use shape to solve “shape discriminations”? *Learn Mem* 2006;13:287–97. [PubMed: 16705141]
- Powers MK, Green DG. Single retinal ganglion cell responses in the dark-reared rat: grating acuity, contrast sensitivity, and defocusing. *Vision Res* 1978;18:1533–9. [PubMed: 726302]
- Prusky GT, West PW, Douglas RM. Behavioral assessment of visual acuity in mice and rats. *Vision Res* 2000;40:2201–9. [PubMed: 10878281]
- Prusky GT, Harker KT, Douglas RM, Whishaw IQ. Variation in visual acuity within pigmented, and between pigmented and albino rat strains. *Behav Brain Res* 2002;136:339–48. [PubMed: 12429395]

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.jneumeth.2009.04.023.

Fig. 1. Schematic of the Floor Projection Maze. (A) A standard LCD projector displayed two-dimensional images on the floor of an open maze via a mirror angled under the maze floor. (B) A top down aerial view of the maze during the contrast sensitivity task. The rat held ready position for 500 ms prior to stimulus presentation; the ready position was marked by the white square outline located under the animal. (C) The rat then made a choice between the correct (stripped) and incorrect (grey) stimulus. Choice was operationally defined as approach to either stimulus. Reward was delivered in the food port located on the South wall of the maze. Note, the clear plexiglas divider inserted between the stimulus pair that forced the animal to go either left or right.

Fig. 2.

Visual stimuli used in each experiment. (A) Each trial consisted of an intertrial interval (ITI) during which a grey floor was projected. Trial onset was indicated by white noise paired with the appearance of a square that marked the “ready position” location. Once animals entered and/or stayed at the ready position for a predetermined time, a stimulus pair was presented in the north (Choice). (B) Three pairs of dice stimuli were used in the easy discrimination, Experiment 1. Stimuli resembled a top down view of the face of a die. The correct stimulus is on the left for each pair. (C) Animals failed to reach criterion on a color discrimination of yellow versus blue. (D) A triangle and square of equal luminance and area were used in the difficult discrimination, Experiment 2. The point of the triangle was oriented toward the

viewing angle. (E) Examples of levels of contrast used to determine contrast sensitivity in the pigmented Long-Evans rats, Experiment 3. Contrast percentage is indicated above the stimulus, top row, or below the stimulus, bottom row. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 3.

Trials to criterion on the Dice Task and the Triangle Task. Normal adult rats ($n = 3$) rapidly learned to discriminate dice pairs to a criterion of 10 consecutive correct trials (*left*). Trials to criterion decreased with each problem with a mean of 83 trials for the first problem (1 vs. 4) to a mean of 17 trials for the third problem (6 vs. 3). When placed on a more difficult discrimination, using a triangle and a square of equal luminance and area, rats reached criterion of 10 consecutive correct trials in a mean of 521 trials (*right*).