

Contextual processing of brightness and color in Mongolian gerbils

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Brightness and color cues are essential for visually guided behavior. However, for rodents, little is known about how well they do use these cues. We used a virtual reality setup that offers a controlled environment for sensory testing to quantitatively investigate visually guided behavior for achromatic and chromatic stimuli in Mongolian gerbils (*Meriones unguiculatus*). In two-alternative forced choice tasks, animals had to select target stimuli based on relative intensity or color with respect to a contextual reference. Behavioral performance was characterized using psychometric analysis and probabilistic choice modeling. The analyses revealed that the gerbils learned to make decisions that required judging stimuli in relation to their visual context. Stimuli were successfully recognized down to Weber contrasts as low as 0.1. These results suggest that Mongolian gerbils have the perceptual capacity for brightness and color constancy.

Introduction

Vision plays an important role for the discrimination and recognition of objects. However, varying illumination conditions can result in drastic changes of intensity and spectral composition of the light reflected from an object. The ability of the visual system to compensate for such influences of illumination, known as brightness and color constancy, is therefore essential for reliable object recognition in varying environments (Figure S1). Humans achieve color constancy by taking into account information from the larger visual context. Specifically, relationships between colors in a scene, i.e., contrasts between object surfaces and their surroundings, are more stable under changing illumination than absolute intensity or spectral composition (Foster, 2011). For example, an object with higher reflectance will always reflect more light compared to a neighboring object with lower reflectance although their absolute intensities will vary with changing illumination. Local and

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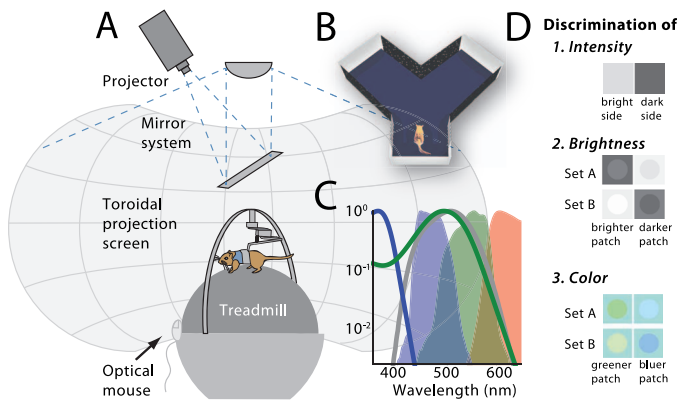


Figure 1. Testing visual discrimination in gerbils. (A) Experimental apparatus. (B) Virtual Y-shaped maze for 2AFC experiments. At trail start, the animal is located in the stem of the Y. Visual stimuli are presented at the end walls of the two arms. The animal responds by walking into the chosen arm. (C) Spectra of gerbil photoreceptors and projector primaries. Blue, green, and gray solid lines show the spectra of gerbil S and M cones and rods, respectively. Colored filled curves represent the spectra of the projection system. (D) Example stimulus sets for the three discrimination tasks. See text for details.

global contrasts thus are cues that contribute strongly to color constancy (Foster, 2011; Hurlbert & Wolf, 2004; Kraft & Brainard, 1999). Studies on the abilities of nonhuman vertebrates to make contextual visual judgments as required for color constancy are rare (Dörr & Neumeyer, 2000; Locke, 1935). In particular, nothing is known so far about whether rodents can use such important second-order visual cues for behavior.

Among rodents, vision is particularly well developed and ecologically important in Mongolian gerbils (*Meriones unguiculatus*), which exhibit a unique receptor configuration (Govardovskii, Röhlich, Szél, & Khokhlova, 1992) and behavior under daylight conditions (Pietrewicz, Hoff, & Higgins, 1982). The Mongolian gerbil's retina is composed of two cone and one rod photoreceptor types (Jacobs & Deegan, 1994; Jacobs & Neitz, 1989). The cones are maximally sensitive at wavelengths around 360 nm (S cones) or around 490 nm (M cones), respectively (Jacobs & Deegan, 1994; Figure 1C). Gerbil M cones show the most prominent short wavelength shift known in mammals with a sensitivity maximum that lies at shorter wavelengths than that of the rods (Jacobs & Neitz, 1989).

Here we introduce an experimental paradigm to investigate visually guided behavior in gerbils. In our experiments, the animals learned to select visual stimuli based on their brightness or color relative to a surrounding background, suggesting that Mongolian gerbils may exhibit brightness and color constancy. In

addition, our data reveal principles of the related task learning dynamics.

Methods

Animals

Experiments were performed with four adult female Mongolian gerbils (*Meriones unguiculatus*). Training started at an age of 8 months, at which the animals weighed between 80 and 90 g. The animals received a diet that kept their weight at about 85%–90% of their free feeding weight. All experiments were approved according to national and European guidelines on animal welfare (Reg. von Oberbayern, AZ 55.2-1-54-2532-10-11).

Experimental apparatus

We used a virtual reality (VR) setup (Figure 1A) for rodents in which the animal was placed on a Styrofoam sphere acting as a treadmill. Movements of the animal induced rotations of the sphere that were detected by two infrared sensors connected to a computer. The computer generated and updated a virtual visual scene that was displayed via a video projector and a mirror system on a projection screen surrounding the treadmill. The distance of the screen from the animal was 65 cm. For real-time rendering, we used Vizard Virtual Reality Toolkit (v3.18, WorldViz, <http://www.worldviz.com/>; for a more detailed description, see Thurley et al., 2014). Calibration of the stimulation apparatus and verification of luminance and chromaticity of individual stimuli was done using a PR-655 SpectraScan® Spectroradiometer (Photo Research, Inc.).

We performed three different visual discrimination experiments (achromatic intensity discrimination, brightness constancy, and chromatic contrast discrimination) using a forced choice paradigm. Visual targets were presented at the ends of the arms of a virtual Y-shaped maze; the other walls of the maze were covered with black-and-white striped and dotted textures (Figure 1B). No other virtual light sources were used to ensure controlled intensity and chromatic contrast of the stimuli. At the beginning of each trial, an animal was located at the end of the virtual Y maze's stem facing its fork (see Figure 1B). The end walls subtended $28^\circ \times 28^\circ$ of visual angle initially and increased in size as the animal approached them in the VR. The animal had to run to the end of the correct arm to receive a food reward (Nutri-plus gel, Virbac, Bad Oldesloe, Germany). In addition, the animal received visual

feedback at the end of each trial. The entire projection screen was either set to black (correct) or to white (wrong) for two seconds (1 and $49 \text{ mW}\cdot\text{sr}^{-1}\cdot\text{m}^{-2}$, respectively). A new trial was initiated by reintroducing the animal at the virtual Y maze's stem. Stimulus presentation was randomized between left and right arms. Each experimental session lasted until the animal had performed at least 20 decisions or, during training, until 15 min had passed. Animals performed one to two sessions per day.

Behavioral training

The animals were accustomed to the VR for about 2 weeks (Thurley et al., 2014). Afterward, the animals performed the actual visual discrimination experiments. For each type of experiment, the animals were first trained with conditions in which high contrasts were used. The training period lasted until the animals had learned the task, i.e., they made correct decisions above chance for at least 3 days in succession. In each experiment, half of the animals were trained for one stimulus condition (e.g., to choose the brighter stimulus); the others were trained for the other condition.

Stimuli

Stimulus differences were quantified by Weber contrast $I_1/I_2 - 1$, where $I_1 > I_2$ represent the intensities of stimuli that have to be discriminated. In the brightness discrimination task, the animals had to discriminate a high-intensity stimulus from a low-intensity stimulus presented at different arms of the Y maze (Figure 1D). Here contrast was defined as contrast between the bright and the dark arm.

For the brightness contrast experiment, the animals had to discriminate the contrasts of stimuli consisting of a central patch on a uniform background at the end of each maze arm. Stimulus patches were circular with a diameter of two thirds of the height of the stimulus wall. In one of the arms, the center patch was of higher intensity than the background; in the other arm, it was darker. Contrast was the same in both cases (Figure 1D). To exclude that animals could solve the task based on absolute intensity, two stimulus sets were used in which the overall intensities were exchanged but the local intensity relationships remained the same (Figure 1D). Average stimulus radiances were $10 \text{ mW}\cdot\text{sr}^{-1}\cdot\text{m}^{-2}$ for the dark stimuli and $42 \text{ mW}\cdot\text{sr}^{-1}\cdot\text{m}^{-2}$ for the bright stimuli. Chromatic contrasts were produced by either increasing the intensity of the green projector primary and decreasing the intensity of the blue primary in the stimulus patch relative to

the background (+G–B stimulus), or vice versa (–G+B stimulus), illustrated in Figure S2. The amounts of changes in each primary were chosen to achieve equally large but opposite cone contrasts in M and S cones. To minimize the possibility of errors due to uncertainties in the spectral shapes of the cone sensitivities in the long wavelength range, the red display primary was not used for the chromatic stimuli. Cone excitations were calculated as the inner products between the display spectra and the gerbil spectral sensitivity functions (Jacobs & Deegan, 1994; see also Figure 1C). To exclude that achromatic cues could be used to solve the task, two stimulus sets were used, in which overall intensities varied but the local chromatic contrasts remained the same (Figure 1D).

Analysis of behavior

We assessed the performance of the animals in two ways: (a) by evaluating the correctness of a decision and (b) to determine stimulus-unrelated influences on decision making, by analyzing which arm of the Y maze the animals took. Because both parameters are binomially distributed random variables, we used binomial tests for significance testing. Confidence intervals were calculated as Clopper-Pearson intervals based on the beta distribution. For differences between proportions, we tested with a chi-squared test. Data analyses were done with Python 2.7 using the packages Numpy 1.7.1, Scipy 0.12, Statsmodels 0.5.0 (Seabold & Perktold, 2010), and Matplotlib 1.3 (Hunter, 2007).

Psychometric analysis

Contrast values were computed as positive numbers, and the sign of the contrast was used to indicate in which arm of the Y maze the rewarded stimulus was placed. Psychometric functions are thus given as percentage of rightward choices as a function of this signed Weber contrast. A negative contrast value indicates that the target stimulus was presented at the left arm; a positive contrast indicates that the target was on the right. We fitted psychometric data with the function

$$\psi(c) = \lambda_l + (1 - \lambda_l - \lambda_r)F(c; m, w) \quad (1)$$

where $F(c)$ is a cumulative Gaussian and $F^{-1}(c)$ its inverse. The variables $\lambda_{l,r}$ represent the lapse rates for leftward and rightward choices, respectively. The parameter $m = F^{-1}(50\%)$ is the mean of the cumulative Gaussian $F(x)$ and determines the left-right bias. The width $w = F^{-1}(1-\alpha) - F^{-1}(\alpha)$ represents the interval over which the psychometric function is growing, i.e.,

a way to parameterize its slope. We set $\alpha = 25\%$ such that w corresponds to the interval $[25\%, 75\%]$. Thus, w can be regarded as a discriminability threshold or as “just noticeable difference.” Because two stimuli were present in the brightness contrast and chromatic contrast experiments, the threshold contrasts reported here slightly underestimate the values that would be obtained for single stimuli. For fitting Equation 1 to the data, we used a Bayesian inference approach that relies on a Markov chain Monte Carlo method (Kuss, Jäkel, & Wichmann, 2005) implemented in the Psignifit 3.0 package (Fründ, Haenel, & Wichmann, 2011).

Probabilistic choice modeling

As an alternative way to describe the behavioral data, we made use of a probabilistic choice model (Busse et al., 2011). The model assumes influences from three different sources on the decision in the current trial: (a) a sensory component $v[c(t)]$ that describes the impact of the contrast stimulus c in trial t , (b) history terms describing the influence of a previous correct $s(t-1)$ or false choice $f(t-1)$, and (c) a general bias b_0 into which all other influences are collapsed, such as a general preference for one arm of the Y maze or a tendency to lapse. The history sequences are mutually exclusive, i.e., for correct trials, $f(t) = 0$ and $s(t) = \pm 1$ if the chosen arm is right or left. Correspondingly, if the decision was wrong, we set $s(t) = 0$ and $f(t) = \pm 1$. In a reduced version of the model, we left out the history terms. All model components were linearly combined into a decision variable

$$z(t) = v[c(t)] + b_s s(t-1) + b_f f(t-1) + b_0, \quad (2)$$

which itself was assumed to determine the probability

$$p = \frac{1}{1 + \exp(-z)} \quad (3)$$

of choosing the right arm by sampling from a Bernoulli distribution.

To derive the parameters $v(c)$, b_s , b_f , and b_0 of the model, we fitted a generalized linear model using Statsmodels 0.5.0 (Seabold & Perktold, 2010) and assumed a binomial distribution family with a logit link function. To assist fit convergence at reasonable values, we restricted the z values to remain within ± 3 via a quadratic penalty term $(|z| - 3)^2$ for $|z| > 3$. We simulated the model by applying the same sequence of presented contrasts as in the experiments and taking the history according to the outcome of the last simulated trial. The probability p from Equation 3 for the simulated z values was then used for binary random sampling. To determine the intervals that contained

95% of the simulation runs in the figures below, we performed 25 to 50 runs.

For better visualization, we fitted the contrast responses inferred from behavior $v(c)$ with a hyperbolic ratio function of contrast (Albrecht & Hamilton, 1982)

$$f(c) = R_0 + R_{\max} \frac{c^n}{c_{50} + c^n} \quad (4)$$

where R_0 is the baseline, R_{\max} the overall responsiveness, c_{50} the semisaturation contrast, and n determines the steepness.

Results

We performed three different visual experiments with Mongolian gerbils (Figure 1D) using a two-alternative forced choice (2AFC) paradigm. For precise control of stimulus presentation and behavioral measurement, we implemented the tasks in VR (Harvey, Collman, Dombeck, & Tank, 2009; Hölscher, Schnee, Dahmen, Setia, & Mallot, 2005; Thurley et al., 2014; Figure 1A, B and Methods).

Training for intensity discrimination

In the first series of experiments, animals had to discriminate stimuli of different intensities (see Figure 1D) that were presented on opposing arms of a virtual Y maze. Half of the animals were rewarded for choosing the arm with the brighter stimulus; the other half were rewarded for the darker stimulus. The intensity difference between the training stimuli corresponded to a Weber contrast of 2. In all gerbils, performance increased gradually over sessions and became significantly different from chance level after about nine sessions, corresponding to between 200 and 300 trials. At this point, performance was at 75% correct or higher (binomial test, $p \leq 0.05$; Figure 2).

To understand choice behavior during task learning, we analyzed the data with respect to which arm of the Y maze the animals chose (Figure S3A). All animals initially showed a strong preference for choosing the Y's left arm. These leftward biases largely disappeared with ongoing training (binomial test, $p > 0.05$ for all animals in the final sessions).

As a more systematic account of choice behavior, we described the learning dynamics using a probabilistic choice model (Busse et al., 2011, and Methods). For the training data, we used a reduced model with only two components: (a) the influence $v(c)$ of the stimulus contrast c and (b) an overall bias b_0 . Choosing the two-parameter variant allowed for fitting the model to each session separately despite the rather low number of

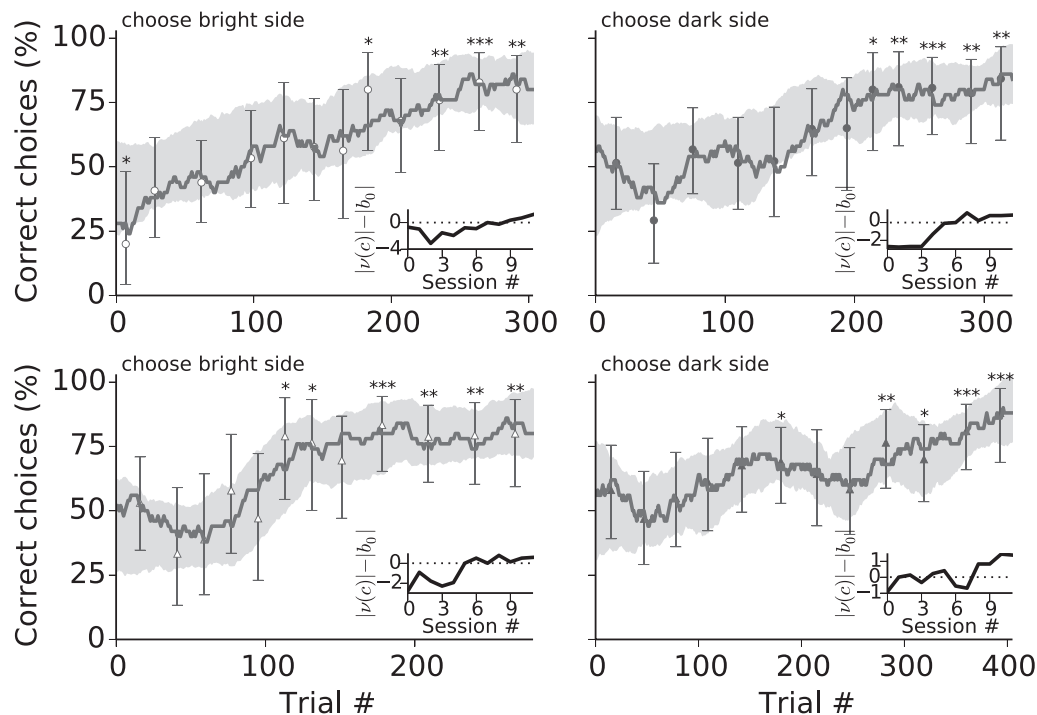


Figure 2. Learning intensity discrimination. Individual learning curves are given for each of the four gerbils. The dark solid lines depict the performance over trials. The curves were calculated with a moving average of 50-trial window size. Markers represent session averages, corresponding error bars are 95% binomial confidence intervals, and stars designate significant difference from chance level according to a binomial test (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$). The animals on the left panels (open symbols) had to choose the brighter stimulus, the animals on the right panels (filled symbols) the darker stimulus. Gray shaded areas delimit intervals that contain 95% of simulation runs with the probabilistic choice model. Insets: Difference between the magnitudes of the two model parameters, the sensory term $v(c)$ and the bias b_0 for consecutive sessions. Layout of the panels and symbols identify data from the same animal throughout the paper.

available data points (on average, an animal performed 27 ± 6 trials per session). The model fits were in good agreement with the data, and the development of the model's parameters over sessions confirmed the above conclusions (Figure 2 insets): Initially the bias term b_0 was large in comparison to the influence of the stimuli $v(c)$, corresponding to strong preferences for one of the maze's arms. During training, the contribution of $v(c)$ increased, and behavior became less and less influenced by the bias b_0 . Finally, choice behavior depended more strongly on the stimulus $v(c)$ than on the side.

From these results, we conclude that gerbils can learn to do intensity discrimination in a virtual 2AFC paradigm. The animals' initial preferences for choosing one of the maze arms was overcome by training, leading to consistent stimulus-dependent choice behavior.

Intensity task

With the trained animals, intensity discrimination was tested for 16 sessions using stimuli with smaller intensity differences. Performance was largely stable for most contrasts from the beginning (Figure S3B). Figure

3 shows the psychometric data such that it takes into account the side at which the target stimulus was presented. The percentage of rightward choices is plotted as a function of contrast with positive contrast values corresponding to target stimuli presented on the right arm and negative contrast values corresponding to target stimuli presented on the left arm.

By fitting psychometric functions to the data, we analyzed choice behavior with regard to (a) stimulus discriminability and (b) influences of side preferences. Accordingly, we derived the following characteristic parameters: (a) a discriminability threshold w that quantifies the contrast interval in which behavioral performance changes over 50% and (b) a side bias m together with the leftward/rightward lapse rates $\lambda_{\Delta r}$ (see Methods for details).

Sensory thresholds w were 0.5 for one animal and between 0.1 and 0.2 for the other three animals. Overall, the animals' performances displayed considerable idiosyncrasies. Two of the animals showed strong left biases, resulting in almost perfect performance when target stimuli were presented on the left (i.e., negative values on the abscissae in Figure 3) but remaining barely above chance for targets on the right. Both animals had lost their left arm preference at the

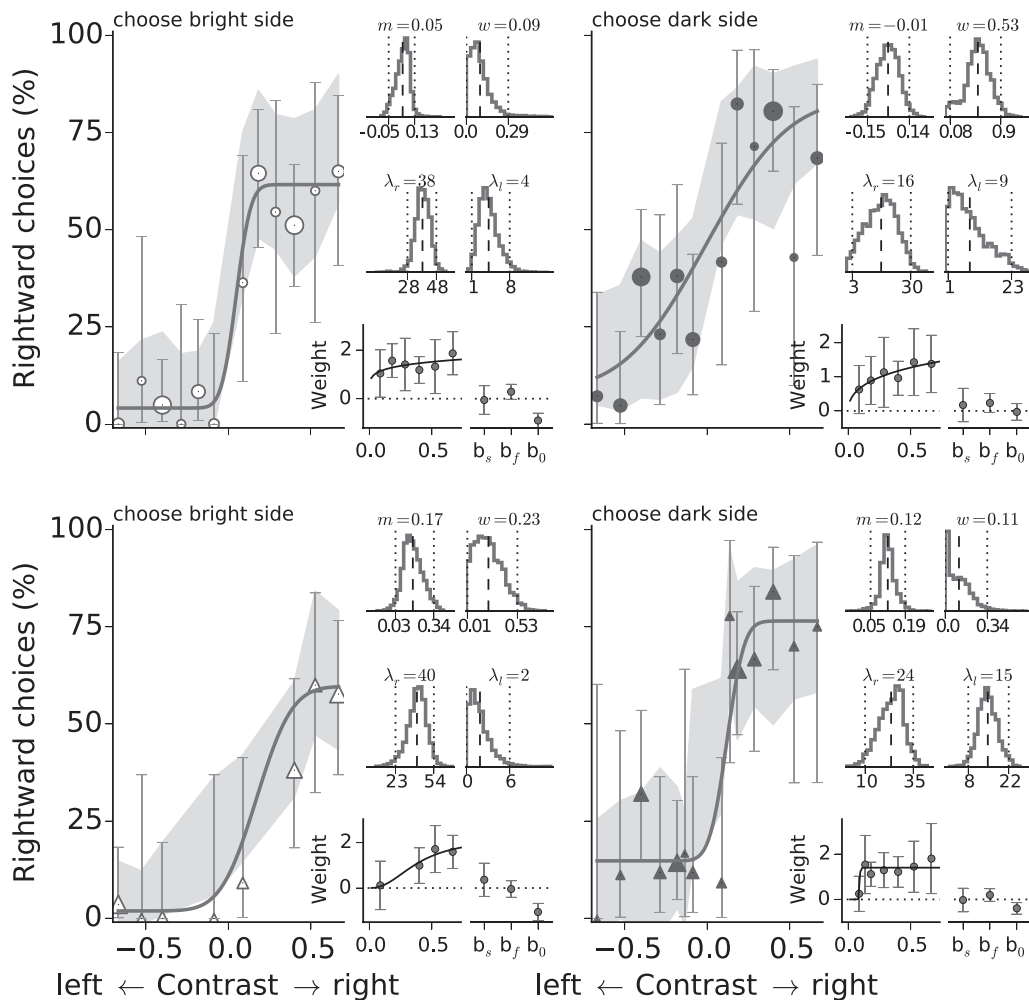


Figure 3. Psychometric results for intensity discrimination. The figure is organized similarly to Figure 2. Psychometric functions for the individual animals are given as the percentage of rightward choices as a function of the contrast between the brighter and the darker stimulus. The size of the symbols is proportional to the number of trials included in the data point. Error bars are binomial confidence intervals. Solid lines are fitted psychometric functions. The gray shaded areas are intervals that contain 95% of single simulation runs with the probabilistic choice model. The four upper small panels to the right of each plot give distributions of parameters of the psychometric functions derived from the Bayesian inference approach. For notation, see main text and Methods. Above each of those panels, the average parameter values are given and indicated with dashed lines. These averages are the estimates used for the fits given in the main panels. Dotted lines give the 95% confidence intervals of the respective parameter. Lower small panels show model parameters with 95% confidence intervals. Solid lines are fits with a hyperbolic ratio function.

end of the training phase with larger stimulus differences (cf. Figure S3A, bottom left). This behavior is in line with a strategy mix that lets the animal choose right only if it is confident that the target is on the right side but suggests going left otherwise. For the other two animals, left arm preferences were less pronounced. Both animals displayed more similar lapse rates for leftward and rightward choices.

The bias m showed a less heterogeneous picture across animals but was in line with a general left preference (Figure 3). In contrast to the lapse rates, the parameter m captures side biases at low absolute contrast.

We also fitted the probabilistic choice model to the psychometric data. The model's stimulus-dependent parameters $v(c)$ were monotonously increasing with contrast, and the overall biases b_0 were in agreement with the psychometric analyses above (Figure 3, bottom inset panels). Arm preferences have different signs in the model's b_0 and the psychometric function's m parameters (i.e., a left bias is represented by $b_0 < 0$ but $m > 0$). Because history parameters describing past successes b_s or failures b_f have been reported to be important for explaining choice behavior (Busse et al., 2011), we included them in the analysis. However, their influence turned out to be negligible given that both

parameters did not differ significantly from zero (bottom-most small panels in Figure 3).

Our VR intensity discrimination paradigm thus allowed for determining psychometric discriminability thresholds. The quantification of choice behavior and performance was consistent between classical psychophysical analysis and probabilistic choice modeling.

Brightness task

We next investigated whether gerbils are able to select stimuli based on their intensity relative to the immediate surround. In this experiment, the animals had to compare stimuli consisting of a central uniform stimulus patch on a background (see Figure 1D). Two of the animals had to choose the side on which the test patch was of higher intensity than its background (brighter stimulus), and the other two animals had to choose the side on which the test patch was of lower intensity than its background (darker stimulus). To exclude that animals could use absolute intensity as a cue, two sets of stimulus pairs with different absolute intensity levels were used (see Figure 1D and Methods) and selected randomly from trial to trial. During training, we used stimuli with Weber contrasts of 0.225.

The task is illustrated in more detail in Figure 4A, in which we provide stimuli and choices of one animal from the last trials of the training for the brightness task. The animal was trained to choose the stimulus that was darker than its immediate surround independent of the stimulus' overall intensity. In the 16 trials given, the animal identified the target stimulus 13 times, and it did not use a strategy based on overall intensity. Similar results were obtained for all of our animals during the training of the brightness task.

During training, the animals' decisions were at chance level for the first 100 to 150 trials (about five sessions). Afterward, performance rather abruptly became significant and saturated at about 75% correct choices (Figure S4A). Again choice behavior initially showed left arm preferences (Figure S4B). At the end of training, performance was similar for both stimulus sets (chi-squared test, $p > 0.2$ for all animals). Because the animals had been trained in the previous experiment to compare the intensities of the stimuli presented at the left and right arms, we reasoned that in the beginning they might try to apply those learned strategies. However, this was not the case. With respect to absolute intensities, the choices were close to chance level and remained like this throughout (Figure S4A). As in the intensity discrimination task, the results were also reflected in the parameters of the reduced probabilistic choice model (Figure S4A, insets).

After training, test stimuli with lower contrasts were interspersed with the training stimuli. To keep the

animals motivated, we began with a test/training stimulus ratio of 0.75. This ratio was subsequently reduced until none of the training stimuli remained. In total, we undertook 19 test sessions with each animal. The psychometric data are shown in Figure 4. Again performance was similar for both associated stimulus sets (chi-squared test, $p > 0.07$ for all animals and contrasts). From the beginning, animals performed at a stable level for each contrast, indicating that they immediately generalized the task from the training stimuli to stimuli with contrasts and intensity levels they had never encountered before (Figure S4C). Biases m as determined from the psychometric functions were relatively low. Similarly, the probabilistic choice model indicated only small biases and history contributions but a substantial contribution of the sensory terms.

All four animals were able to discriminate stimuli down to a contrast of 0.1 or below. These results demonstrate that gerbils are able to select visual stimuli based on relative brightness cues.

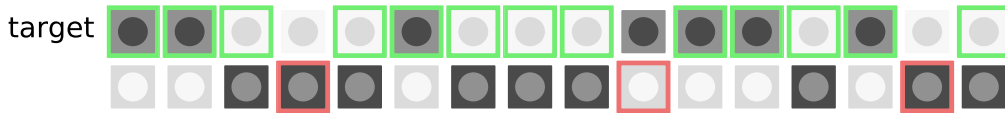
Color task

In a final series of experiments, we tested the gerbils' ability to select color stimuli based on color contrast relative to the background. Two of the animals had to choose the side on which the test patch color was shifted toward the green projector primary relative to the background (+G–B stimulus); the other two animals had to choose the side on which the test patch color was shifted toward the blue primary (–G+B stimulus). Again two sets of stimuli with different absolute intensity levels were used to exclude that animals could use intensity, achromatic contrast, or absolute cone excitation as cues. Furthermore, we assigned the +G–B and –G+B tasks to the animals such that the two animals with the darker target in the achromatic contrast experiment were assigned different chromatic targets, and likewise the two animals with the brighter target in the achromatic contrast experiment were assigned different chromatic targets.

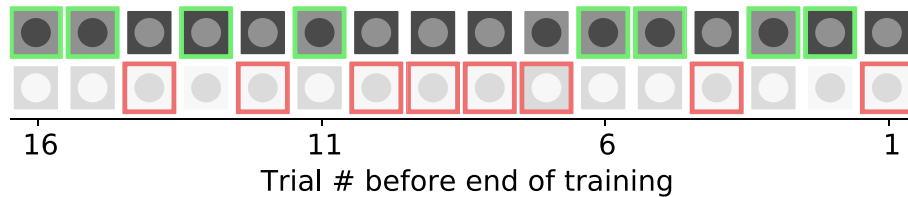
For training, we used stimuli with chromatic contrasts of 0.5. Again performance was at chance level initially and gradually increased with training (left panel of Figure S5A). After about 200 trials (five sessions), all animals achieved close to 75% correct choices. Performance was not different for the different stimulus sets (chi-squared test, $p > 0.17$ for all animals). A strategy based on achromatic cues was not adopted by the animals (Figure S5A). Choice behavior was influenced by maze arm preferences early in training, similarly as in the previous experiments, and to some extent remained throughout the training in two animals (Figure S5B).

A

Correct strategy: Choose patch that is darker relative to its background



Hypothetical alternative strategy: Select patch with lower overall intensity



B

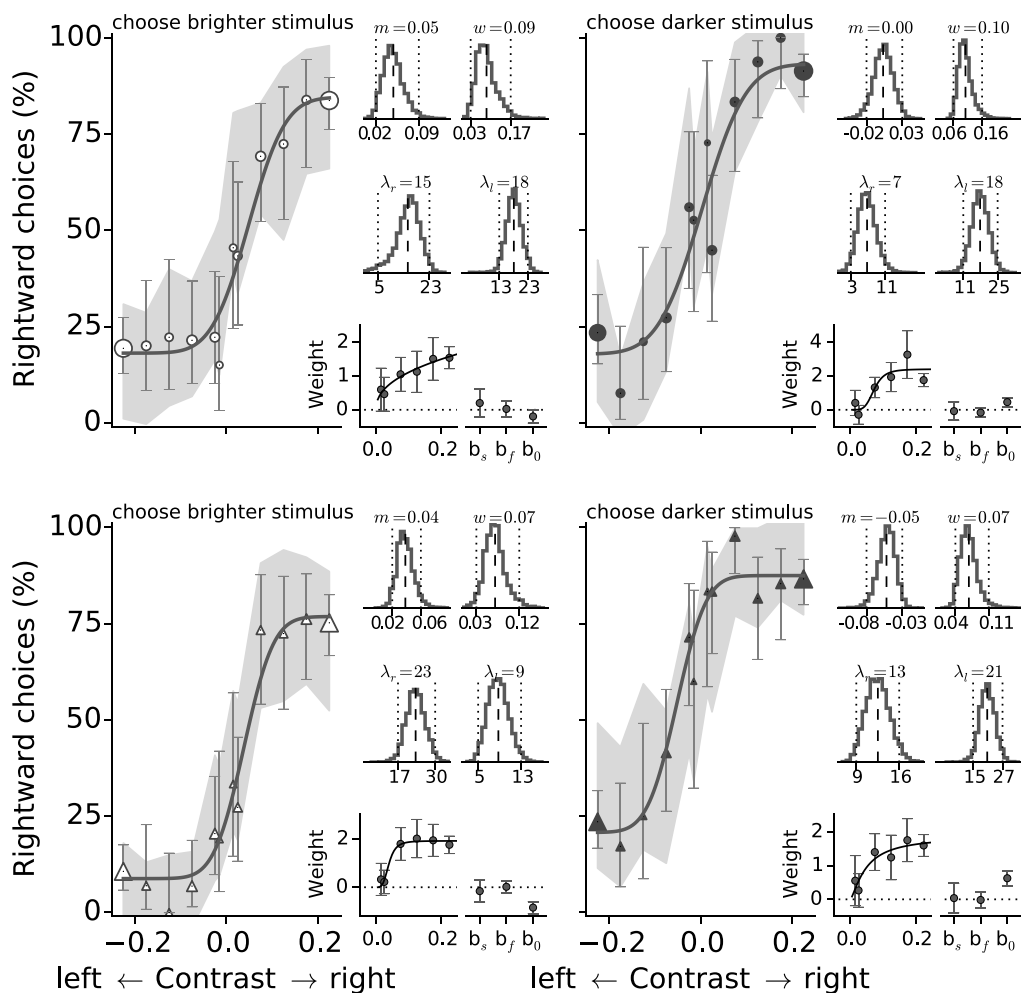


Figure 4. Psychometric results for the brightness task. (A) Final trials in the training phase of the brightness task for an example animal (bottom right in B). Upper panel: Stimulus pairs are given for each trial with targets in the upper and distractors in the lower row. The animal's choices are indicated with green (correct) and red (false) frames around the stimulus in the trial. Note that, in the experiments, target and distractors were presented on left and right arms of the maze at random. Lower panel: Same data as above but plotted corresponding to what would be expected if the animal had used a strategy based on overall intensity. (B) Psychometric

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functions for the brightness task. The figure is composed similarly to Figure 3. Psychometric data for the individual animals is given as the percentage of rightward choices as a function of the contrast between the central patch and its local background (symbols and error bars represent averages and 95% confidence intervals, respectively). Solid lines are fitted psychometric functions. The gray shaded areas correspond to the probabilistic choice model, whose parameters are given in the bottom-most of the small panels to the right. The four upper small panels to the right of each plot give distributions of parameters of the fitted psychometric functions. Averages and 95% confidence intervals are highlighted. Solid lines are fits with a hyperbolic ratio function.

After training, we conducted seven test sessions. Again test stimuli of lower contrast were interspersed with increasing rate into the set of training stimuli. Figure 5 shows the results of the psychometric analysis. Performance was not significantly different for the two stimulus sets (chi-squared test, $p > 0.06$ for all animals and contrasts), and the animals immedi-

ately performed at a stable level for each contrast (Figure S5C). As in the previous experiments, two of the animals showed arm preferences (Figure 5, lower panels). The psychometric data of all animals were consistent with the probabilistic choice model. The contrast parameter v was monotonously increasing with contrast c , and the general bias term b_0 was

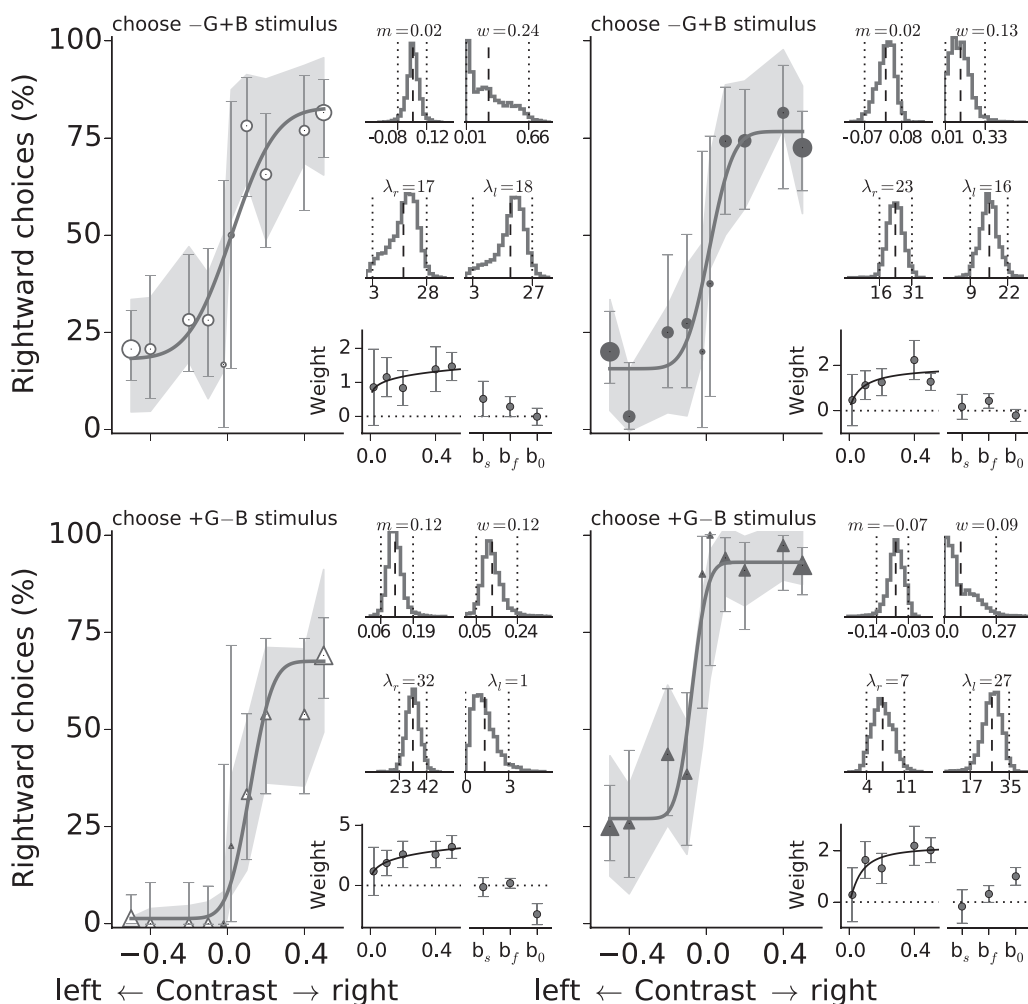


Figure 5. Psychometric results for color discrimination. The figure is composed similarly to Figure 3. The animals in the first row had to choose the $-G+B$ patch, the animals in the second row the $+G-B$ one. Psychometric data for the individual animals is given as the percentage of rightward choices as a function of the chromatic contrast (symbols and error bars represent averages and 95% confidence intervals, respectively). Solid lines are fitted psychometric functions. The gray shaded areas correspond to the probabilistic choice model, whose parameters are given in the bottom-most of the small panels to the right. The four upper small panels to the right of each plot give distributions of parameters of the fitted psychometric functions. Averages and 95% confidence intervals are highlighted. Solid lines are fits with a hyperbolic ratio function.

significant for the two animals that showed arm preferences. The history parameters b_s and b_f were again negligible. All animals could discriminate stimuli down to contrasts between about 0.1 and 0.2. These results demonstrate that gerbils are able to select visual stimuli based on relative color cues.

Discussion

In this study, we investigated the ability of Mongolian gerbils to perform brightness and color judgments. The behavioral tests were implemented using a VR setup for rodents (Thurley et al., 2014).

Intensity and contrast discrimination

To establish our psychophysical approach, we began with a simple intensity task. The results demonstrated the ability of gerbils to discriminate stimuli by intensity. However, for object vision under natural conditions, judging stimuli in relation to their visual context is more relevant (Foster & Nascimento, 1994). We therefore investigated whether gerbils are able to distinguish stimuli based on contrast to their background regardless of absolute intensity. To ensure that the animals' choices depended on brightness contrast, we used stimuli with varying intensities, such that the only indicator for reward in the stimuli was the achromatic contrast between stimulus patch and background. The animals successfully learned to choose the correct stimuli, indicating that gerbils can take context into account when using brightness cues for behavior. Finally, we tested whether gerbils are able to identify stimuli based on chromatic contrast regardless of intensity. As with achromatic contrasts, the animals learned to choose the correct stimuli, indicating that gerbils can use contextual color cues for behavior. Because brightness and color relationships between surfaces in a scene are strong cues to brightness and color constancy (Hurlbert & Wolf, 2004; Kraft & Brainard, 1999), the results presented here provide a first indication that gerbils are capable of such perceptual constancies.

With our paradigm, it is conceivable that animals would not choose the stimuli based on local brightness or color relationships but instead learn which of the two stimuli was rewarded for each stimulus pair separately. However, in the testing sessions, the animals' performance was above chance and at a stable level for each contrast from the very beginning. This corroborates the conclusion that the gerbils immediately generalized the task from the training stimuli to

stimuli with contrasts and intensity levels they had never encountered before (Figures S3B, S4C, S5C).

Quantitatively, the thresholds measured psychophysically in our experiments were slightly lower than increment thresholds determined from electroretinogram measurements in gerbils as reported in previous studies (Jacobs & Deegan, 1994). Thresholds for achromatic and chromatic contrast discrimination tended to be lower than for intensity discrimination. This could be expected because, for intensity discrimination, intensities had to be compared across the arms of the maze whereas, in the contrast tasks, the primary comparison was between the stimulus patches and their immediate background.

Stimuli in our experiments were generated using a standard projector designed for human vision. Such a system achieves only marginal stimulation of gerbil S cones, whose sensitivity range lies at much shorter wavelengths than those of human S cones. Thus, although the relative differences, i.e., contrasts, were the same for S cones and M cones in the experiments, overall stimulation was estimated to be orders of magnitude lower for S cones than for M cones (Table S1). Nevertheless, the animals were able to make the spectral discriminations. It is conceivable that the gerbil S cones are actually more sensitive at their long-wavelength tails than indicated by the published spectral sensitivity curves (Jacobs & Deegan, 1994). Those curves, derived from templates (Dawis, 1981) going back to the Dartnall (1953) nomogram, provide accurate estimates of spectral sensitivity around the peak, but are notoriously unreliable for estimating the tails (Dawis, 1981). In particular, for spectral sensitivity curves peaking in the short-wavelength range, the width tends to be underestimated (Dawis, 1981). Moreover, the long-wavelength tail of the gerbil S cone log spectral sensitivity is just an extrapolation by a straight line (Jacobs & Deegan, 1994), which is a very coarse approximation. Even small changes in the slope of this line lead to substantial increases in the estimates of S cone stimulation. It is therefore not unlikely that S cone stimulation in our experiments was actually higher than estimated based on the published spectral sensitivity curves.

Alternatively, a contribution of rod signals, which is feasible in dichromat color vision (Kremers & Meierkord, 1999; Montag & Boynton, 1987; Reitner, Sharpe, & Zrenner, 1991), could underlie the discrimination performance of the animals. In any case, the performance exhibited by the gerbils in our experiments demonstrates the ability of these animals to make judgments based on relative spectral composition of the stimuli.

Given potential uncertainties in the cone spectral sensitivities, a concern could be that our estimates of cone excitations were imprecise such that the color

stimuli we used actually also contained brightness cues. We therefore calculated how far the M cone spectrum would have to be shifted toward longer wavelengths to make the bright $-G+B$ stimuli darker than the dark $+G-B$ stimuli, in which case it would have been possible to solve the task based on brightness cues. We found that such an intensity inversion would require a shift of the M cone sensitivity by more than 30 nm, which we consider unrealistic.

Further evidence that discrimination was based on spectral content and not brightness is provided by the initial behavior of the animals in the color task. In the preceding experiment, two of the animals had learned to choose the brighter stimulus; two had learned to choose the darker stimulus. If a brightness cue existed in the chromatic task, that is, either $-G+B$ or $+G-B$ were correlated with brightness, one would expect that two of the animals would have directly been able to solve the task. However, all animals had to relearn (Figure S4A), confirming that they could not rely on brightness to solve the task.

Behavioral analysis

Our approach allows for detailed analyses to determine psychophysical properties such as discriminability thresholds and lapse rates for the specific tasks. In trained animals, thresholds were comparable across individuals and tasks. Lapse rates did not differ strongly, indicating that the difficulty of the tasks was similar for all animals. We described the psychometric data using two different approaches: (a) psychometric function fitting (Kuss et al., 2005; Wichmann & Hill, 2001) and (b) choice modeling (Busse et al., 2011; Carandini & Churchland, 2013; Gold, Law, Connolly, & Bennur, 2008). Lapse rates depended on the arm on which the target stimulus was presented and could be attributed to biases due to preferences for choosing one of the arms in our maze. This was revealed because we tested over a rather wide range of contrasts that always included stimuli at which performance saturated. Therefore, arm preferences dominated the stimulus-independent contributions to choice behavior in our experiments and may thus explain why previous choices had negligible effects on the current choice, compared to what has been reported by others (Busse et al., 2011; Lau & Glimcher, 2005).

Analyzing the learning dynamics for the individual tasks, we were able to show how the initial preferences for one side of the Y maze across all animals disappeared with learning but reappeared when stimuli became harder to differentiate (i.e., at lower contrasts), indicating that the animals may have applied different strategies depending on their confidence about the stimulus. Such biases between influence from sensory

cues and internal preferences are well known for choice behavior (Busse et al., 2011; Gold et al., 2008).

VR with rodents

Since the first reports of successful application of VR for rodents (Dombeck, Khabbaz, Collman, Adelman, & Tank, 2007; Hölscher et al., 2005), VR setups became very popular. This popularity is due to the fact that VR setups allow for the use of advanced recording techniques in behaving animals, such as intracellular recordings (Domnisoru, Kinkhabwala, & Tank, 2013; Harvey et al., 2009) or optical imaging of populations of neurons (Harvey, Coen, & Tank, 2012; Keller, Bonhoeffer, & Hübener, 2012). The behavioral paradigms in use, however, are usually very limited compared to what is standard in psychophysics even with rodents (Carandini & Churchland, 2013). Nevertheless, 2AFC tasks were implemented before with rodents on a treadmill (Harvey et al., 2012; Thurley et al., 2014), but so far, no psychometric data were measured. The present study is the first that successfully determined discrimination thresholds in VR with rodents.

Conclusions

Brightness and color constancy and contextual influences on neural processing as potential underlying mechanisms have been investigated in primate (Locke, 1935; Wachtler, Sejnowski, & Albright, 2003) and nonprimate mammalian species (MacEvoy & Paradiso, 2001) but so far not in rodents. Our results show that Mongolian gerbils can perform visually guided behavior that requires judgments of stimuli in relation to their visual context and thus provide first evidence for the capability of brightness and color constancy in rodents.

Moreover, with the present study, we presented a psychophysical paradigm that can be used with rodents to investigate perceptual performance in behaviorally relevant tasks.

Keywords: virtual reality, gerbil vision, color vision, perceptual constancy

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