Behav Res (2012) 44:919–923
DOI 10.3758/s13428-012-0219-8

Design of a noninvasive face mask for ocular occlusion in rats and assessment in a visual discrimination paradigm

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Published online: 16 June 2012

Abstract The rat visual system is structured such that the large (>90 %) majority of retinal ganglion axons reach the contralateral lateral geniculate nucleus (LGN) and visual cortex (V1). This anatomical design allows for the relatively selective activation of one cerebral hemisphere under monocular viewing conditions. Here, we describe the design of a harness and face mask allowing simple and noninvasive monocular occlusion in rats. The harness is constructed from synthetic fiber (shoelace-type material) and fits around the girth region and neck, allowing for easy adjustments to fit rats of various weights. The face mask consists of soft rubber material that is attached to the harness by Velcro strips. Eyeholes in the mask can be covered by additional Velcro patches to occlude either one or both eyes. Rats readily adapt to wearing the device, allowing behavioral testing under different types of viewing conditions. We show that rats successfully acquire a water-maze-based visual discrimination task under monocular viewing conditions. Following task acquisition, interocular transfer was assessed. Performance with the previously occluded, "untrained" eye was impaired, suggesting that training effects were partially confined to one cerebral hemisphere. The method described herein provides a simple and noninvasive means to restrict visual input for studies of visual processing and learning in various rodent species.

Keywords Monocular occlusion · Vision · Learning · Behavior · Rodents

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In recent years, the rodent visual system has become an important model to study mechanisms of experience-dependent plasticity during early development, as well as adulthood. For example, monocular deprivation, initially shown in kittens to result in profound ocular dominance shifts in the primary visual cortex (V1) (Hubel & Wiesel, 1970), now provides an important tool to characterize molecular and cellular mechanisms of synaptic development and plasticity in rats and mice (Hofer, Mrsic-Flogel, Bonhoeffer, & Hübener, 2006; Jiang, Huang, Morales, & Kirkwood, 2005; Smith, Heynen, & Bear, 2009). Furthermore, the development of sophisticated visual tasks allows a detailed analysis of the perceptual abilities of rodent species (Prusky, Alam, & Douglas, 2006; Prusky, West, & Douglas, 2000).

Initially, relatively long-term monocular deprivation was used to examine the effects of visual experience (or the lack thereof) on V1 development (Hubel & Wiesel, 1970). In addition, procedures to temporarily restrict the input to one eye through monocular occlusion are useful for identifying training-induced plasticity in specific, anatomically restricted parts of the rodent nervous system. For the latter, it is important to note that the anatomical organization of the rodent visual system displays a high degree of lateralization. That is, the large majority (>90 %) of retinal ganglion axons cross to the contralateral lateral geniculate nucleus (LGN) and V1 (Cowey & Perry, 1979; Lund, Lund, & Wise, 1974; Sefton, Dreher, & Harvey, 2004), resulting in a strongly lateralized activation of the contralateral hemisphere under monocular viewing conditions. Chang and Greenough (1982) demonstrated that rats undergoing maze training under monocular viewing conditions experience a greater increase in dendritic spines in V1 contralateral to the open eye, an observation consistent with the high degree of anatomical lateralization of the rat visual system.

In previous work on rodents, techniques to restrict visual input to one eye have involved adhesive eye patches (Adelstein & Crowne, 1991; Crowne, Forsyth, & Fitzgerald, 1994), lid suturing (Chang & Greenough, 1982; Prusky et al., 2006) or enucleation (Prusky et al., 2006; Shinohara et al., 2012). Problems with adhesive patches remaining in place have been reported (Adelstein & Crowne, 1991). Lid suturing and enucleation both necessitate surgical procedures under general anesthesia and are either nonreversible, or require a further surgical procedure to open the sutured eye. Given these limitations, alternative methods of restricting visual input in small rodent species might offer advantages over previously used techniques.

Here, we provide a description of the construction and use of a simple, noninvasive harness–face mask device that allows for temporary occlusion of one or both eyes in rats. Behavioral data for the acquisition of a water-maze-based discrimination task under monocular viewing conditions are provided, demonstrating that rats successfully acquire visual tasks using this device. Further behavioral tests performed with the previously occluded, "untrained" eye suggest that there is relative little interocular transfer of information for this type of visual discrimination learning in rats.

Materials and method

Subjects

Experimental procedures were conducted on 23 adult (400–700 g) male Long–Evans rats (Charles River Laboratories, Inc., St. Constant, Quebec, Canada). The animals were singly housed in a colony room (12/12-h reversed light cycle; light on at 7:00) with free access to food and water. All behavioral procedures took place during the day (dark cycle), typically between the times of 10:00 and 17:00 h. All experiments were conducted in accordance with published guidelines of the Canadian Council on Animal Care and approved by the Queen's University Animal Care Committee.

Harness and face mask construction

The apparatus consists of two parts, a harness and face mask.

Harness The harness was constructed of 1-cm-wide, synthetic cloth material (obtained from lanyards or shoelaces) approximate 50 cm in length, knotted in an adjustable figure-8 design using a simple overhand knot (Fig. 1). The two loops fit snugly around the rat's upper body (just behind the forelegs) and neck, which allowed the harness to be adjusted to fit animals of different sizes.

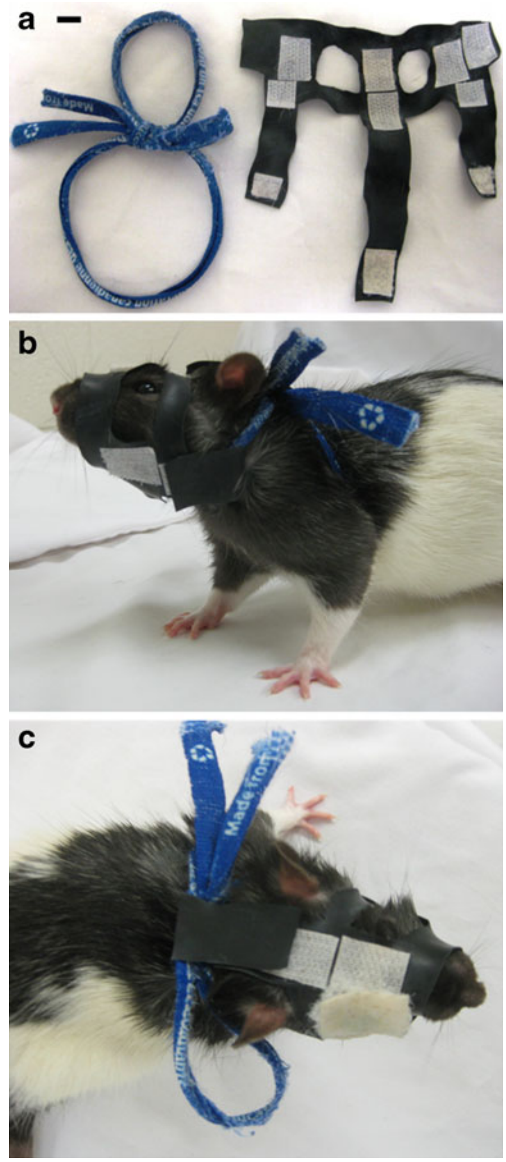

Fig. 1 The harness and face mask designed for monocular viewing. The harness and face mask laid out flat (**a**) (scale bar = 1 cm) and being worn by a rat (**b** and **c**). **a** The small top loop of the harness fits around the neck and behind the ears of the rat. The large bottom loop of the harness fit just behind the rat's forelimbs and around the girth region. (**b** and **c**) The face mask wraps around the rat's head, attaching to itself under the chin. The three long straps of the mask are used to secure it to the harness, with the longest (middle) strap placed centrally on the top of the head and the two lateral straps placed on the sides of the head. The straps are wrapped around the harness and folded back onto themselves and secured with Velcro pieces. (**c**) View of the top of the head, showing an eye patch in place, which can readily be switched between the two eyes

Face mask The face mask (3 cm wide × 10.5 cm length) was cut from soft rubber material obtained from dishwashing gloves. Adhesive Velcro strips were attached to the mask (Fig. 1), allowing it to be securely wrapped around the animal's head, leaving the eyes, ears, and nose unobstructed. The eye holes were approximately 1.5–2 cm in diameter, with hooked Velcro placed around each hole, allowing soft Velcro eye patches (rectangular, 2 × 1.5 cm) to be attached to the mask, thereby closing the eye holes. Eye patches could easily be attached and removed without obvious discomfort of the animal. The face mask attached to the harness by three rubber and Velcro straps (1.5 cm wide, 4–7 cm length), one on each side of the head/neck and one along the top of the head (Fig. 1). Velcro was positioned so that the soft, nonhooked side faced the animal's body, thus avoiding discomfort and skin irritation. Experimenters monitored the rat's breathing, movement, and vocalizations to ensure that the mask and harness fit each animal properly. This device was worn during habituation and subsequent visual discrimination training.

Visual discrimination performance under monocular viewing conditions

The performance of rats wearing the harness–face mask device was assessed using a visual discrimination procedure conducted in a Y-shaped water maze apparatus described previously (Hager & Dringenberg, 2010). In this task, rats were required to distinguish two distinct visual cues mounted at the ends of the two Y-maze goal arms, separated by a central divider (50 cm in length). The visual cues were two white sheets of paper (28 × 21.5 cm) with three equally spaced black bars (length 15 cm, width 3 cm, spaced 3 cm apart, black area 135 cm^2) in either a vertical or horizontal orientation. Cues were mounted at the ends of the goal arms 1 cm above the water surface and 50 cm away from the point where rats were required to enter one of the two goal arms. One of the visual cues was paired with the location of a hidden escape platform (P+), while the other indicated the absence of the platform (P–; the cues and designations were counterbalanced across different animals). The location of the escape platform (and the associated cues) changed randomly from trial to trial during acquisition training (Hager & Dringenberg, 2010). All rats were fitted with the face mask and one eye patch to create monocular viewing conditions (n = 10 and n = 13 for right and left eye occlusion, respectively).

Prior to the start of discrimination training, rats were handled and habituated to wearing the harness and face mask for five consecutive days (10 min/day). Subsequently, monocular discrimination training was carried out as described previously (Hager & Dringenberg, 2010), with the following modifications: Habituation Day 1: Rats swam in the Y-maze (20 trials) while wearing the harness and were required to find the hidden platform without the use of discrete visual cues to indicate platform location. Habituation Day 2: Animals were again required to swim (20 trials), but now wore the harness and the face-mask with one eye occluded. Furthermore, visual cues (P+ and P–) were now present at the ends of the goal arms to indicate the platform location.

Following these habituation procedures, the formal training phase was initiated, which occurred over multiple days (ten trials/day) and continued until a preset performance criterion was reached (8/10 correct trials per day for three consecutive days). During training, rats were required to form an association between the visual cues and platform location under monocular viewing conditions. For each trial, the platform (and the associated visual cues) were pseudorandomly placed in one goal arm, with the restriction that the platform was assigned to each goal arm for five out of the ten daily trials.

Probe trial test of interocular transfer

Following task acquisition, five additional test days were given (ten trials/day). For two of these trials (randomly assigned, with the stipulation that they could not occur consecutively or on the first of the ten daily trials), the eye patch was reversed from the previously closed "untrained" to the open "trained" eye to test for interocular transfer of the learned information. For these "probe" trials, the platform was removed from the maze and the first arm entered by the animal was recorded as their correct or incorrect choice.

Data analysis

The data were expressed as means ± standard errors of the means (*SEM*s) and were evaluated by analyses of variance (ANOVAs) using SPSS software (v. 19.0, SPSS Inc., Chicago, IL). Probe trial data were assessed by comparing the percentages of correct responses between the open "trained" eye and the previously closed "untrained" eye within subjects and across the five probe trial days using repeated measures ANOVA.

Results

Task acquisition

A total of 23 rats were trained on the visual discrimination task wearing the harness and face mask to create monocular viewing conditions. The rats were randomly assigned to receive training with either the left (n = 13) or the right (n = 10) eye. Furthermore, the visual cues were also counterbalanced so that half of the animals were given vertical and horizontal bars as P+ and P–, respectively (i.e., indicating the platform location and absence), with the remaining rats receiving the opposite association. Importantly, all rats readily adjusted to wearing the mask without obvious signs of distress or discomfort.

Of the 23 trained rats, 16 successfully reached the preset training criterion (at least 8/10 correct trails for three consecutive days). Rats that did not meet this criterion after 20 training days were excluded from further analysis. The average time to reach the criterion and successfully discriminate the visual cues was 12.1 days (range 7–18; see Fig. 2a). Whether vertical or horizontal bars were used as P+did not affect the number of days to reach the performance criterion for successful task acquisition, $F(1, 15) = 0.3$, $p > .05$. Similarly, there was no significant difference between rats trained with the left or the right eye on the number of days to acquire the task, $F(1, 15) = 0.01$, $p > .05$.

It is of interest to note that task acquisition under monocular viewing conditions was delayed relative to acquisition using binocular vision described in previously published work using the same apparatus and training procedure (Fig. 2a; binocular data adapted from Hager & Dringenberg, 2010). This observation indicates that binocular information appears to aid in visual discrimination performance assessed under the present test conditions.

Test of interocular transfer

Following task acquisition, five additional test days were given, each consisting of eight regular trials (trained eye open) and two randomly inserted probe trials, where the eye patch was reversed to allow testing with the previously occluded, "untrained" eye. As is shown in Fig. 2b, performance with the "trained" eye (M = 89.2 %) was consistently better across the five test days relative to the untrained eye [M = 66.3 %; main effect of eye, $F(1, 60) = 16.9$, $p = .001$]. Furthermore, there was no significant change in performance over the five test days [main effect of day, $F(4, 60) = 1.7$, $p > .05$] and no interaction between test eye and probe trial day, $F(4, 60) = 0.190$, $p > .05$. These results indicate that there is relatively little interocular transfer for the type of visual information acquired under the present training conditions.

Discussion

The results summarized above demonstrate that rats readily learn to discriminate visual cues under monocular viewing conditions created by the harness and face mask described here. However, the time to acquire the task under monocular conditions appears to be longer than that required with binocular training (mean of 12.1 days, range 7–18 days, in the present study vs. a mean of 9 days, range 6–13 days, in Hager & Dringenberg, 2010, for binocular training). Consequently, it appears that the task becomes more challenging when visual input is restricted to only one eye.

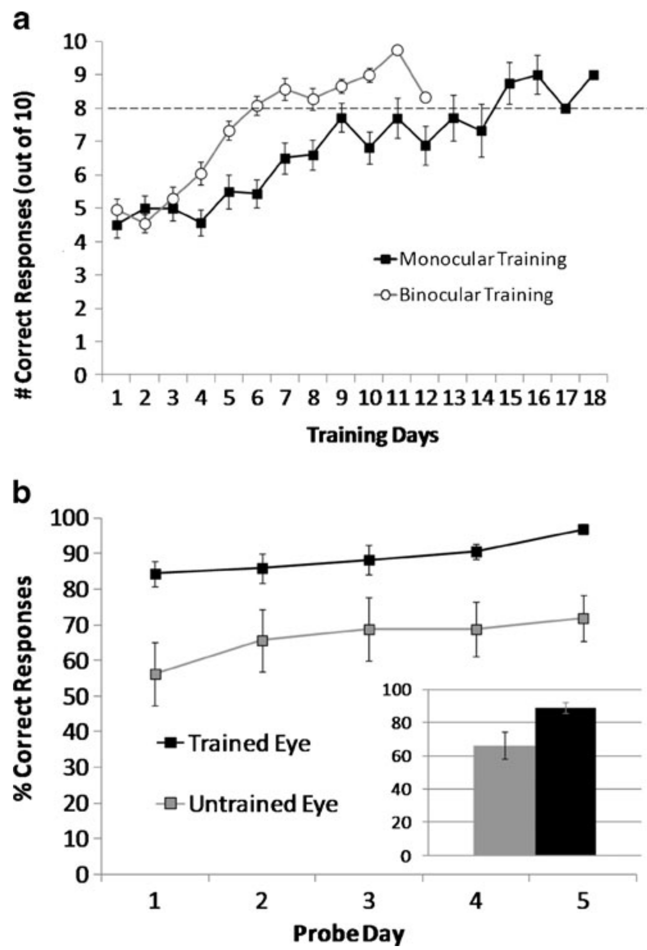

Fig. 2 Monocular visual discrimination performance. (**a**) Visual discrimination performance for animals trained under binocular (n = 24) and monocular (n = 16) viewing conditions (average ± *SEM* correct responses in the Y-maze are shown). The dashed line indicates the criterion for successful completion of the task. Note the faster task acquisition for binocularly trained animals. The binocular data are adapted from (Hager & Dringenberg, 2010). (**b**) Monocularly trained animals were administered five additional training days (ten trials/day). For two of these trials, the eye patch was switched to the previously nonoccluded, trained eye. Note that performance was significantly impaired for the untrained eye, indicative of weak interocular transfer of visual information acquired during training ($p < .05$). The insert bar graph shows significantly ($p < .05$) different averages for correct responses across the five test days for the trained (M = 89.2 %) and untrained (M = 66.3 %) eyes

An advantage of the mask designed here is the ease of switching the eye patch between the two eyes, thus allowing for an uncomplicated study of interocular transfer of visual information. The noninvasive and relatively stress-free nature of the eye patch devised here clearly offers advantages over alternative methods of restricting visual input, such as adhesive eye patches (Adelstein & Crowne, 1991; Crowne et al., 1994), lid suturing (Chang & Greenough, 1982;

Prusky et al., 2006), or enucleation (Prusky et al., 2006; Shinohara et al., 2012), especially when quick switching of the patch between the two eyes is required during behavioral testing. In addition, masks to limit visual input might also be useful for investigations necessitating longer-term ocular occlusion, for example in the context of developmental studies. While the use of such masks has been described for kittens (e.g., Mitchell, Kind, Sengpiel, & Murphy, 2006), it appears that similar techniques have not been employed with rodents. Consequently, the mask described here may also benefit investigations requiring prolonged periods of visual restriction.

Interestingly, monocularly trained rats displayed only weak interocular transfer of previously learned information, as determined by probe trials testing the previously occluded, “untrained” eye. This observation may suggest a high degree of hemispheric lateralization of learned information despite extensive training over many days. Alternatively, it could be that the “untrained” eye does not have access to the representation of the visual information that was clearly acquired and stored during training. Given these findings, it appears that additional, invasive surgeries to further disconnect the cerebral hemispheres (e.g., cutting the optic chiasm or corpus callosum; e.g., Adelstein & Crowne, 1991; Chang & Greenough, 1982; Crowne et al., 1994; Shinohara et al., 2012) are not required to achieve a highly lateralized training effect, at least for the behavioral paradigm used in the present investigation.

Questions of hemispheric specialization for various types of information and cognitive abilities have received considerable attention over the last decades (Gazzaniga, 2005). Rodents constitute a useful model to address at least some of these questions regarding the specialized roles of individual hemispheres, particularly in light of the extensive crossing (and anatomical separation) of visual inputs between retina and V1 (Cowey & Perry, 1979; Sefton et al., 2004). Interestingly, there appears to be an increased use of rodents for research examining perceptual, developmental, and cognitive aspects of visual processing, as well as the synaptic mechanisms mediating these phenomena (Hager & Dringenberg, 2010; Hofer et al., 2006; Prusky et al., 2006; Prusky et al., 2000; Shinohara et al., 2012; Smith et al., 2009). The apparatus described here can benefit studies by introducing a technique that allows for the easy, inexpensive, and stress-free restriction of visual input in rodents in the context of behavioral training paradigms.

Author note The research described here was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).

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